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A *Giardia* parasitic in a Bursate Nematode living in the Viscacha.

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The material for these observations was kindly given to me by Dr. T. Goodey, of the Institute of Agricultural Parasitology. While examining the contents of the small intestine of a viscacha (*Viscacia viscacia*) which died at the London Zoological Gardens he found and recognised a heavy infection with free living flagellates of the genus *Giardia* in the lumen of the intestine of a bursate nematode (*Viannella sp.*).

An examination of the literature of the genus *Giardia* shows that hitherto no helminth has been found parasitized by it.

In 1923 Lavier described a new species of the flagellate *Giardia viscaciæ* from the intestine of the viscacha (*Viscacia viscacia*) a rodent from South America. The fact that the nematode *Viannella*, found parasitized by a free *Giardia sp.*, was obtained from the intestine of the viscacha makes it probable that the worm had become infected by the same flagellate through the habitat in which it was situated.

In the description and discussion which follows it will be observed that this protozoon, found in very large numbers parasitic in the intestine of the nematode *Viannella sp.*, is morphologically indistinguishable from *Giardia viscaciæ* Lavier, 1923, found in the intestine of a viscacha.

Description of the Flagellate found free in the gut of the bursate nematode *Viannella sp.*

Examination of fresh living material under $\frac{1}{6}$ inch and $\frac{1}{12}$ inch objectives revealed the lumen of the nematode's intestine filled with actively motile flagellates. These were seen free in the lumen of the gut or attached to the epithelial lining of it by their sucker like depressions. They were so numerous as to suggest a culture. No cysts could be

detected and further, a search of the fæcal contents of the gut of the viscacha in which the nematodes were found in large numbers failed to demonstrate any examples of the genus *Giardia* either free or encysted. Hundreds of the worms were present and every specimen examined was heavily parasitized with the flagellates. The activity of the protozoa and the heaviness of the infections furnished undoubted proof that these were living and multiplying as true parasites in a suitable habitat, namely the nematode's gut.

Fixed and Stained Material.

The intestinal contents of the worms were smeared on coverslips and fixed wet in Bouin's picro-formol solution and finally stained with Heidenhein's iron hæmatoxylin.

In length they varied from 11μ to 16μ and in breadth 6μ to 8μ. About 13μ in length was most frequent. The ratio of length to breadth was most frequently about 1·8. No measurements were made on living specimens.

A comparison of these dimensions with those given by Lavier for *Giardia viscaciæ* in specimens fixed and stained by the same method show that the free flagellates in our case were smaller, but the ratio of length to breadth was approximately the same.

Lavier's measurements of *G. viscaciæ* were: Length 13μ to 18μ and. most frequently 16μ. Breadth $6{\cdot}5\mu$ to 12μ but mostly about $8{\cdot}5\mu$. The proportion of length to breadth varied from 2·1 to 1·5 but was mostly 1·8.

The parabasal bodies in many were absent or unstained (vide text fig. 1 but figures 2 and 3 illustrate two and one of these bodies respectively).

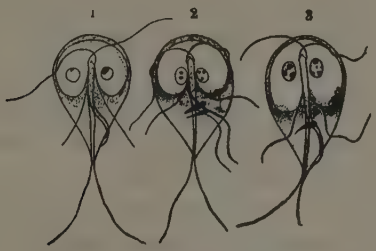
In shape these are elongated broad at one end and tapering at the other. Comparing those with Lavier's figures of *G. viscaciæ* there is a striking similarity.

The disposition of the chromatin in the nuclei varied in different specimens as illustrated in the text figures 1, 2 and 3. Morphologically except in size we were unable to differentiate specific differences from those described by Lavier. As the host of the worm died we were unfortunately unable to carry out further observations. The relation-

ship of the *Giardia sp.* found by us in the *Viannella sp.* could not be established with *Giardia viscaciæ* in the intestine of the viscacha as all examinations failed to demonstrate either free forms or cysts of this flagellate in the fæcal contents of the dead host of the parasitized nematode.

Moreover, no infection occurred in the numerous specimens of *Trichostrongylus sp.*, a closely allied nematode, found in the same habitat.

A second Viscacha died later and again all the specimens of *Viannella* were heavily parasitized, but no free forms or cysts could be found in the intestinal contents or in the associated *Trichostrongylus* nematodes.



Figs. 1-3. *Giardia sp.*, magnified approx. 1333 diam.

Discussion regarding various species of the Genus *Giardia*.

Specimens of this genus have been described and named in mammals, birds, reptiles and amphibians. To distinguish species on morphological grounds is in many cases unsatisfactory. Slight variations in size, shape, ratio of length to breadth and the character and outline of the parabasal bodies have been used.

Kofoid and Christiansen (1915) showed differences in morphology between *Giardia microti* of field-mice and *Giardia muris* of rats and mice. More recently Hegner (1924) has diagrammatically demonstrated the specific differences of these flagellates in man and some of the lower animals.

Lavier (1923) notes that it is possible to group the various named species into three types according to the ratio of length to breadth, namely, the human type, the rat or mouse type, and the frog or tadpole type. Lavier emphasizes however the necessity of biological experiments. If a certain species can infect one host only, its specificity is definitely established.

Various observers have, however, obtained contradictory evidence regarding infection with *Giardia* from animals to man and *vice versâ*. Fantham and Porter (1916) reported the successful infection of kittens and mice with the human flagellate, but Simon (1922) failed to infect laboratory rats from man and concludes that human infections are not contracted from rats and mice. Grassi (1881) failed to infect himself from animals, and Moritz and Hölzl (1882) failed to produce a human infection from mice. Stiles claims to have infected a guinea pig from man.

It is thus obvious that cross infection experiments have given rise to a certain amount of confusion. It is, however, generally accepted that the species in rats and mice are identical.

The great difficulty in conducting cross infection experiments of a conclusive character lies in the fact that it is almost impossible to exclude hidden infections in the experimental animals used.

TABLE OF VARIOUS SPECIES OF *GIARDIA* DESCRIBED AS PARASITES OF ANIMALS.

A. Mammals. Man, *Giardia intestinalis* (Lambl, 1859) Alexieff, 1914. Rabbit (*G. duodenalis* Davaine, 1875) Hegner, 1922. Rats and Mice, *Giardia muris*, Grassi (1879), Bensen, 1908. Dogs *Giardia canis*, Hegner, 1922. Guinea Pigs *G. caviæ*, Hegner, 1923. Field Mice *G. microti*, Kofoid and Christiansen, 1915. (Synonym ? *G. pitymysi* Splendore, 1920). Viscacha *G. viscaciæ*, Lavier, 1923.

B. Birds. Falcon (*Elanus cæruleus*) *G. sanguinis*, Gonder, 1911. Heron (*Ardea cinerea*) *G. ardeæ*, Nöller, 1920. (Kotlán, 1922, described *Giardia sp.* in intestine of two birds *Lanius collurio* and *Recurvirostra avosetta*, which might be *G. ardeæ*.)

C. Reptiles. Lizard (*Varanus niloticus*) *G. varani* Lavier, 1923.

D. Amphibians. Frogs and Tadpoles *G. agilis*, Künstler, 1881 (Syn. *G. alata*).

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Observations on *Gastrocystis gilruthi*, a parasite of Sheep in Britain.

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In 1910 Professor Gilruth of the University of Melbourne published a note on the finding of a protozoal parasite in the mucous membrane of the abomasum of sheep in Tasmania, together with a brief description of its morphology. Subsequent to this discovery, Professor Gilruth sent preparations of the parasite to Professor Mesnil of Paris, which led to the discovery of the same organism parasitic in almost all sheep and goats examined at the Paris abattoirs. Investigations were carried out by M. Edouard Chatton, who, during the same year, published a full description of the occurrence, morphology, and probable affinities of the parasite, to which he gave the name of *Gastrocystis gilruthi*. In 1912 Gilruth reported the presence of the cysts in the intestinal wall of certain wild Australian mammals, where it was associated with enteritis.

As a careful search of current literature has revealed no further mention of this parasite, the purpose of the present paper is to confirm and supplement the observations of E. Chatton and to give an account of some experiments which have been undertaken in an attempt to further elucidate its life-history and systematic position.

Incidence.

In November, 1924, Mr. E. A. Lewis, B.Sc., Field Officer of the Institute of Agricultural Parasitology stationed at Aberystwyth, reported to Professor Leiper the presence, in the stomachs of sheep killed there, of cysts of *Gastrocystis gilruthi*, similar to the cysts containing larval nematodes. As it seemed desirable to ascertain the percentage of

British sheep in which this infection occurred, material was then obtained from the London slaughter-houses and a series of systematic examinations was carried out to determine the occurrence of the parasite.

Cysts of *Gastrocystis gilruthi* were found to be present in a very high percentage of the sheep killed, for out of 138 stomachs selected at random, 127 contained parasites of a sufficient size to be readily distinguished on macroscopical examination, *i.e.*, 92 per cent. of the stomachs examined. As it seems probable that the earliest developmental stages are too small to be visible without microscopic examination, the actual percentage of infection may be assumed to be even higher.

No evidence of seasonal incidence has so far been discovered, and unfortunately no data as to the origin of the sheep could be obtained. It was found, however, that by classifying the stomachs according to their degree of infection, as negative, light, medium, etc., with very few exceptions all the material examined on a single day fell under approximately the same class. This seemed to indicate that the occurrence of the parasite varied, being common in some parts of the country and scarcer in others. It is also possible that some breeds of sheep are more commonly infected than others. It is hoped that greater facilities for examination in the future will lead to the solution of these problems.

Habitat.

The cysts occur only in the true stomach or abomasum, where they lie embedded in the mucosa. Cysts have never been found in the intestinal walls, and examinations of the œsophageal portions of the stomach have always given negative results, although the possibility of an infection occurring in these parts cannot definitely be excluded owing to the extreme difficulty of making a thorough examination. On two occasions, in very heavy infections, cysts were found free in the lumen of the stomach and the upper portion of the small intestine. It is possible, however, that these had been dislodged from the mucosa in the handling and washing of the stomachs, as, in subsequent examinations of the surface of the mucosa and contents of unwashed specimens, no free cysts were found.

In heavily infected cases the cysts were present in the proportion of about one per square centimetre of the mucosa, throughout which they

were irregularly scattered. Frequently they were grouped in clusters of four or five within an area of about two square centimetres. In some cases only two or three cysts were found in a whole stomach, and no evidence of a previous heavier infection was shown, while in others, although few cysts were present, the whole of the mucosa was dotted with small crater-like eminences marking the position of former cysts. It is significant that stomachs which contained these marks were always found to harbour a few cysts, often in early stages of development.

Macroscopic Examination.

The appearance of the infected mucosa was found to vary. In the majority of cases the cysts were clearly visible, as small whitish bodies embedded in the centre of semi-transparent, opalescent eminences of the mucosa. Frequently the cysts were obscured by slight hæmorrhagic lesions occupying the summits of these eminences, while in a few cases the character of the mucosa appeared unchanged, being only slightly raised in small rounded patches, thus revealing the presence of the parasite, which could be found on dissection.

The cysts were either rounded or ovoid, varying in size up to 0·9 mm. in their longer diameter. No cysts of less than 0·3 mm. diameter were found. The smallest cysts of 0·3 mm. to 0·4 mm. diameter were always spherical, while the larger cysts which had attained a more advanced stage of development tended to become more and more ovoid. This was probably due to increased pressure following the growth of the parasite.

By exerting a slight pressure on the adjacent mucosa unripe cysts were readily extruded whole, but those cysts which were fully mature, containing fully developed spores, burst on the application of the slightest pressure, pouring forth the spores, which appeared as a milky fluid, on the surface of the mucosa.

Pathology.

Examination of sections cut through the infected mucosa showed that the eminences were due chiefly to the presence of the cysts. These were constantly arranged with their long axis parallel to the gastric glands, and were sunk a distance of from 0·3 mm. to 0·7 mm. below the surface of the mucosa, thus causing a protuberance. In the tissue

surrounding the cysts there was a small-cell infiltration. This was especially marked in those parts immediately subjacent to the cyst. There was also a certain amount of œdema, and this, in conjunction with the infiltration was possibly the cause of the semi-translucent, opalescent appearance of the mucosa as seen macroscopically. There was a slight degeneration of the surrounding epithelium accompanied by congestion. The hæmorrhages previously noted as occurring in the region of some of the cysts was the result of rupture of small capillaries due to congestion caused by the presence of the parasite and consequent pressure.

The crater-like eminences so frequently present in infected abomasa were sectioned and examined. In some of these the remains of collapsed cyst-walls were present, surrounded by a mass of phagocytic cells as described and figured by Chatton. In other cases, however, no trace of the cyst wall could be found, but a narrow cleft was present, leading from the centre of the leucocytic mass to the free inner margin of the mucosa. This cleft was never found where the remains of the cyst wall occurred, and seems to indicate further the possibility of whole cysts being extruded into the lumen of the stomach.

The Cyst Wall.

The cysts when removed from the mucosa were of a dull white colour. The cyst wall, which was apparently composed of a single cell, was a somewhat complicated structure. It could be best studied in the smaller specimens where the contents did not completely fill the cyst and in which no pressure was exerted on the wall. In these young cysts freshly extracted and examined in normal saline solution, the wall was 36μ in thickness, excluding the outer brush-like layer. In older specimens containing almost fully ripe spores the wall was only $7{\cdot}5\mu$ in thickness. This thinning of the cyst wall was obviously due to growth and the increased pressure of the contents, as intermediate stages of development gave intermediate measurements for the thickness of the wall.

The structure of the wall appeared to vary with the stage of development and consequent thickness. Two primary layers were constantly present. Of these, the inner layer was a dark greyish colour, composed of delicate circumferentially arranged fibres in a very finely granular

matrix. This layer formed about one quarter of the whole thickness of the young cysts, and one third in cysts containing spores. The outer layer, which was lighter in colour, was coarsely granular on its inner surface, the granules gradually diminishing in number to leave the outer surface clear and hyaline. The outer hyaline portion was thrown into slight folds and ridges, and gave rise to a peculiar brush-like structure, probably of a rhizopodal nature. Between the inner and outer layers of the wall a distinct light coloured zone could be made out in the youngest cysts, in which it was equal in thickness with the inner layer. With the growth of the cyst and consequent progressive thinning of the wall, this zone became markedly contracted, appearing only as a fine line in the mature stages of development. The brush-like outgrowth arising from the hyaline portion of the outer layer, consisted of short, hair-like processes, about 18μ in length, and apparently served the double purpose of attaching the cyst to the mucosa and increasing its absorptive surface. The arrangement of these processes was found to vary slightly. In most cases they formed a uniform fur-like outgrowth over the whole surface of the cyst, but occasionally, particularly in the young stages, they arose in groups, radiating outwards and giving the appearance of being arborescent structures.

The nucleus of the cyst wall was large, circular or oval when seen in surface view, and of a bi-convex lenticular shape. It varied in size from 80 to 100μ by 40—60μ, and was situated between the inner and outer layers of the wall, causing a considerable thickening where it occurred. The depth of the nucleus was about 20μ in fresh specimens, but was difficult to measure with accuracy. The nuclear membrane was distinct and about 1·5μ in thickness in fresh cysts. The most conspicuous feature of the nuclear structure was the presence of from 15 to 30 large masses resembling nucleoli. In the fresh specimen these masses were of a yellowish hyaline nature. The remainder of the nucleus consisted of a finely granular network.

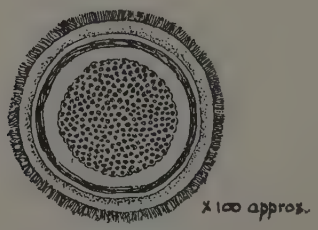
Sections of cysts stained with Ehrlich's Acid Hæmatoxylin and Eosin gave the following colour reactions for the cyst wall. The inner fibrillar layer stained blue, the middle layer, when present, a fainter blue, and the outer layer with its rhizopodal outgrowths was coloured pink by the Eosin. The nuclear membrane, nucleoli and granular network of the nucleus took a very intense blue. Considerable shrinkage took place in

material fixed in Schaudinn's fluid, so that the above measurements could not be confirmed by comparison with the sections.

Contents of the Cyst.

The contents of the cyst were studied both in fresh and stained smears and in sections of the infected mucosa. In the youngest cysts found, of which, unfortunately, no sections have been obtained, the contents were composed of a spherical plasmodial mass. This was suspended in the centre of the cyst, separated from the wall by a distance of about 20μ, this space being presumably filled with fluid. The nuclei of the

Text-fig. 1.—Section through nucleus of cyst wall, showing nucleoli.



Text-fig. 2.—Young cyst, showing structure of wall and plasmodium.

plasmodium were very numerous and more or less regularly spaced throughout the cytoplasm. Those situated marginally formed slight projections in the cytoplasm, giving to the mass a mulberry-like appearance. The intervening cytoplasm appeared to be finely granular.

Development of the Cyst.

The subsequent mode of development is as follows. Increase in the size of the plasmodium takes place, probably accompanied by an increase

in the number of nuclei, until the cyst is completely filled. Further growth results in the enlargement of the cyst and the consequent stretching and thinning of the cyst-wall. Meanwhile the nuclei become arranged around the periphery of a series of cytoplasmic spheres, varying greatly in size. The smallest of these spheres contained only a single nucleus, while the largest are about 80μ in diameter and contain up to 50 nuclei in a single optical plane. A fluid substance fills in the spaces between these masses, which, on the application of slight pressure, revolve within the cyst with a flowing movement. Spore formation then takes place. Radiating outwards from the surface of the sphere, small cytoplasmic projections appear, corresponding in number with the circumferentially arranged nuclei. These increase in size at the expense of the general cytoplasm, and gradually incorporate the nuclei to which they are attached, giving rise to elongated spindle-shaped bodies which remain for some time attached to the reduced central mass of cytoplasm. This central mass appears to be coarsely granular, and is ultimately either absorbed by the developing spores or broken down into the fluid matrix in which the spores become free.

The Spores.

The spores are elongated, slightly curved bodies, rounded at one end and slightly tapering at the other. They are enclosed in a delicate pellicle and remain for some time floating in the fluid contents of the cyst. When mature they measure approximately 12μ in length by $2{\cdot}5\mu$ in breadth. One end remains bluntly rounded or cone-shaped, but the other is prolonged to form a rostrum-like process, often having the appearance of being truncated at the tip. The body is curved, flattened and frequently slightly twisted.

Along the middle of each flattened surface a dark line is visible apparently indicating a slight ridge. The nucleus is faintly visible in unstained spores situated close to the rounded end. More centrally placed is a large highly refractile granule surrounded by a clear area or halo. This is constantly present in all spores. The remainder of the cytoplasm is more or less homogeneous, containing only a few granules smaller in size than that previously mentioned, irregularly scattered and varying in number from one to six.

Examination of freshly extruded ripe spores in a 10 per cent. acetic acid solution deeply tinged with Thionin Blue, showed that a delicate transparent envesting cuticle is present, completely surrounding the body. The internal structure was studied in smears stained with Heidenhain's Iron Hæmatoxylin and Ehrlich's Acid Hæmatoxylin and Eosin. The nucleus is oval, 3μ long by 2μ diameter. It is of the vesicular type with a large, usually eccentrically placed karyosome, and large dots of chromatin rather irregularly spaced, lining the nuclear membrane. The large granule previously mentioned stains intensely with chromatin stains. It is $0{\cdot}75\mu$ in diameter and is situated in a clear halo near the middle of the body and either centrally or on the margin. The other granules present in the cytoplasm are scattered irregularly and are smaller in size and usually without a halo; they also stain intensely. In the rounded end beyond the nucleus, a faintly staining dot is constantly present.

Affinities of Gastrocystis gilruthi.

As the shape of the mature spores, especially the presence of the median ridge on each of the two flattened surfaces, was somewhat suggestive of a bivalve structure of the spore pellicle, the possibility that *Gastrocystis gilruthi* was in the nature of a microsporidian parasite had to be considered, and accordingly attempts were made to cause the spores to extrude a polar filament. Kudo's methods were tried; gentle pressure was exerted on the surface of the coverglass and also the spores were treated with hydrogen peroxide. No filament could be detected either by direct or dark ground illumination.

Again, the structure of the immature cyst at the plasmodial stage of development was not unlike certain developmental stages of *Rhinosporidium seeberi* as described by Ashworth in 1922. Micro-chemical tests were therefore carried out to determine the nature of the cyst wall and of the pellicle enclosing the spores. Both fresh specimens and wax sections were treated with iodine-potassium iodide solution and sulphuric acid, and with zinc-chlor-iodine solution, but no cellulose reactions resulted.

The structure of the deeply staining granule in the body of the mature spore suggested affinities with the *Binucleata*.

Attempts were made to cultivate the spores with a view to discovering their further development ; various media were used for this purpose. Tubes of Locke's Egg medium, Noguchi's nutrient agar medium, Novy-MacNeal-Nicolle medium, and a liquid modification of this, were inoculated with both mature and immature spores. These were incubated at various temperatures, but, owing to the impossibility of excluding bacterial contamination, changes in the reaction of the media were constantly taking place. In order to minimise this the spores were transferred to fresh tubes every other day. In some tubes the reaction was made decidedly acid by the addition of hydrochloric acid.

Daily examinations of the spores in these media were carried out. The immature spores after 36 hours' cultivation gave an erroneous impression of having attained maturity. This was due to the accentuation of the twist caused by shrinkage of the contents within the pellicle. This caused the narrower end of the body to be seen edgewise, giving the appearance of a rostral-like process. A similar accentuation of the twist took place in the mature spores, but no other change was observed until five days after inoculation, when the spores lost their clearly defined outline owing to the disappearance of the pellicle. The loss of the pellicle was followed by a progressive series of changes. First a small clear bubble-like mass appeared, either at the rounded, or more usually, at the pointed end of the spore. This increased in size, and became gradually shifted to one side of the spore which curved over as if to surround it. The nucleus became more and more difficult to make out, but the large refractile granule retained its identity and remained apparently unchanged. The lesser granules gradually disappeared with the nucleus. The cytoplasmic body of the spore remained distinct from the clear hyaline protuberance, which it partly surrounded up to the end of the sixth day in the majority of cases; it then began to lose its outline and merge into the central mass. At the same time the refractile granule began to lose its distinct outline. This process continued and ultimately the spore was represented only by a spherical hyaline mass, free from any granular inclusions, which finally disintegrated on the eighth or ninth day.

These modifications in the spore closely resemble the changes described by J. P. MacGowan as taking place in spores of *Sarcocystis tenella* in

one per cent. Glucose solution. Spores of *Gastrocystis gilruthi* were accordingly placed in 1 per cent. glucose and examined over a period of three hours. During the time the changes described above were exactly reproduced. It is noteworthy, however, that no granules derived from the cytoplasm and due to the breaking up of the nucleus were distinguishable in the ultimate sphere. It seems probable that the rapidity of the degenerative changes in 1 per cent. glucose solution was due to the change in osmotic pressure.

Comparisons between the cysts and spores of *Gastrocystis gilruthi* and *Sarcocystis tenella* show that considerable morphological disparities exist, but the almost universal occurrence of these two parasites in sheep, together with the occasional pathogenicity of the latter point to the possibility of *Sarcocystis tenella* being an aberrant form of *Gastrocystis gilruthi* due to the accidental passage of spores from cysts in the abomasum into the blood stream, and so to the skeletal and cardiac muscles. This is supported by the findings of Von Betegh and Doreich, who fed ducks on *Sarcocystis tenella* obtained from sheep and found early developmental stages of a cystic nature in the muscles of the stomach. A series of feeding experiments is now being carried out on laboratory animals with a view to elucidating this suggestion, the results of which will be published separately.

Transmission.

As regards the mode of transmission of *Gastrocystis gilruthi* practically nothing is known, but it seems probable that it is contaminative. Ripe spores were kept in the intestinal contents, moistened by the addition of normal saline solution. These were found to remain unchanged for three or four days, after which period evidence of internal shrinkage appears in the twisting of the body. Spores kept thus were found to diminish in number and ultimately to disappear. This was thought to be due to the numerous coprozoic organisms which appear in the fæces and which probably preyed upon the spores.

That automatic re-infection may take place in a single animal by spores which remain adhering to the mucous membrane, is indicated by the extremely heavy infections which sometimes occur and by the fact

that abomasa which show signs of previous infection can by careful examination always be found to harbour a few cysts, these being frequently at early stages of development.

In conclusion, I should like to thank Professor Leiper, through whose kindness the material was obtained, and Dr. J. G. Thomson, Director of Protozoology, London School of Hygiene and Tropical Medicine, for the great assistance they have given in the carrying out of these observations.

Plates.

Figs. 1-4.—Mature spores stained with thionin blue in 10 per cent. acetic acid solution, showing investing pellicle. (x2,000.)

Figs. 5-9.—Mature spores stained with Heidenhain's Iron Hæmatoxylin, showing structure of nucleus and cytoplasmic granules. (x2,000.)

Figs. 10-16.—Spores after five days' incubation in culture medium, showing the twisted body. (x2,000.)

Figs. 17-24.—Successive stages of degeneration of spores in culture medium. (x2,000.)

Fig. 25.—Microphotograph of section of immature cyst, showing the arrangement of the nuclei within the plasmodium at the commencement of spore-formation, and the small-celled infiltration in the adjacent mucosa. (x270 approx.)

Fig. 26.—Microphotograph of section of mature cyst, showing the spores and the structure of the cyst wall. (x1,150 approx.)

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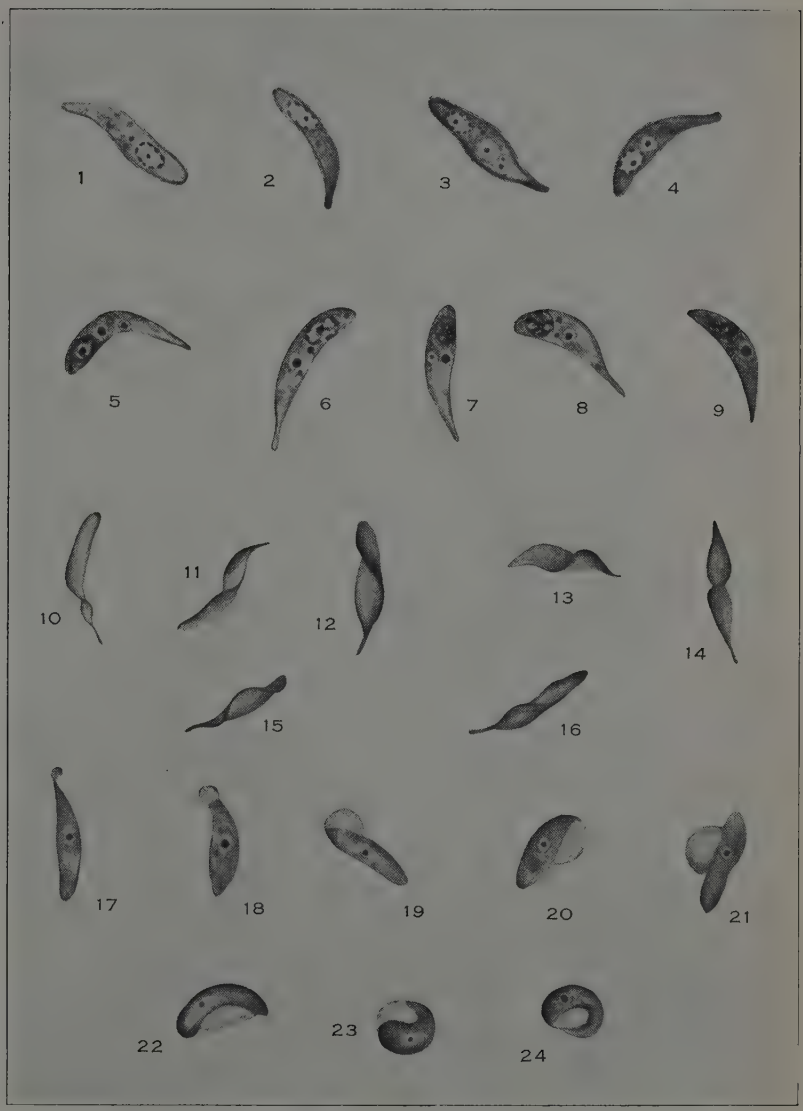

Figs. 1–24.—Spores of *Gastrocystis gilruthi*.

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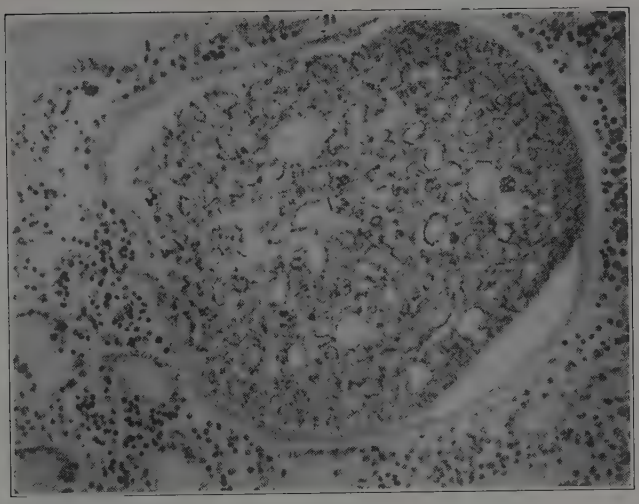

Fig. 25.—*Gastrocystis gilruthi*, section of immature cyst.

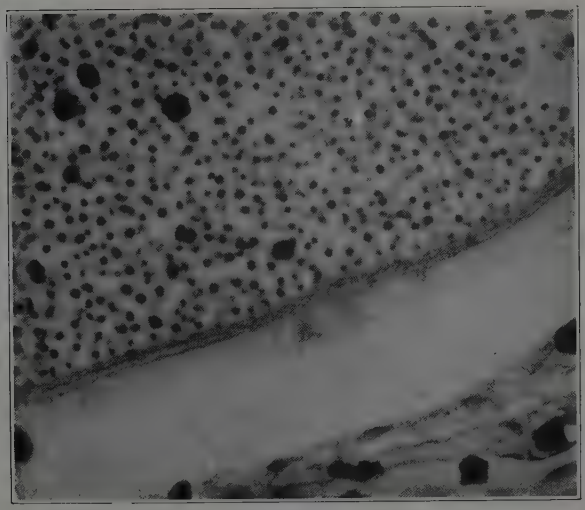

Fig. 26.—*Gastrocystis gilruthi*, section of mature cyst.

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Observations on Two New Species of *Coccidia* parasitic in Snakes.

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The fæces of various reptiles and amphibia living in the gardens of the Zoological Society of London, have been collected for some time past, for investigation by the staff of the Institute of Agricultural Parasitology. An examination of the same material for evidence of protozoal infection has led to the discovery of a number of parasitic protozoa, specially the oöcysts of various Coccidia, including an *Eimeria* occurring in the fæces of African and Indian Pythons and a *Cryptosporidium* in the fæces of a rattlesnake *Crotalus confluentus*.

The following paper has been written under the direction of Dr. J. G. Thomson, in the Department of Protozoology, London School of Hygiene and Tropical Medicine. I am also indebted to Professor Leiper for access to the material.

1.—The *Eimeria* Parasitic in *Python sebæ*.

On two occasions when these fæces were available for examination the oöcysts were found to be present in great numbers. They were oval in shape and varied in size between wide limits. On the first occasion they ranged from 17μ to 36μ in the long axis and from $11 \cdot 5\mu$ to 21μ in the short axis. All intermediate sizes were found, the average dimensions being $27 \cdot 5\mu$ by 17μ. On the second occasion, three months later, the oöcysts were again very numerous, but were much more uniform in size; neither very large nor very small forms were found and the average size was $26 \cdot 2\mu$ by $14 \cdot 7\mu$.

The cyst wall consisted of three layers. The outer layer was formed by a very delicate membrane which completely surrounded the cyst. The middle layer was double contoured, varying in thickness from $0 \cdot 5\mu$ in the smallest cysts, to $0 \cdot 8\mu$ in the largest. It was yellowish in colour and composed of a transparent hyaline substance. The inner layer was a delicate colourless membrane lining the cyst.

The micropyle was very conspicuous and was situated at one pole of the oöcyst. It varied in width from $2 \cdot 5\mu$ to $3 \cdot 5\mu$ and was proportionate with the size of the oöcyst.

In freshly passed fæces the oöcysts were found at various stages of development. In the earliest stage the coarsely granular cytoplasm completely filled the lining membrane of the cyst and no nucleus was visible. In the majority of individuals the cytoplasmic contents of the oöcyst had rounded up into a central spherical mass in which the nucleus was sometimes visible, as a light-coloured, homogeneous mass, more or less marginally situated in the cytoplasmic sphere. Other oöcysts showed various stages of division of the cytoplasm up to the complete separation of four irregularly ovoid sporoblasts. The size of the sporoblasts and of all subsequent stages of development varied according to the size of the containing oöcyst. The nuclei of the sporoblasts were completely masked by the granular nature of the cytoplasm.

Development.

In fæces kept at room temperature without the addition of any moisture development of the oöcystic contents took place. A small proportion of the oöcysts remained with the cytoplasm rounded up into a single central mass. In these no further development took place and ultimately degenerative changes set in. It was, therefore, presumed that in these individuals fertilisation had not been accomplished.

Owing to the different stages of development at which the oöcysts were passed, maturity was not attained simultaneously, but the majority of individuals, *i.e.*, those which were passed with the contents rounded up into a single mass, contained fully formed sporozoites on the 7th day of observation.

The course of development was as follows: With the formation of the sporoblasts, the cytoplasm became slightly more homogeneous. A delicate membrane, the wall of the sporocyst was then secreted around each mass. A slight increase in the thickness of this wall took place, and a peculiar cap-like structure appeared at one end. This cap-like structure was darker in colour and apparently thicker than the remainder of the wall, from which it was very clearly delimited. The sporocysts, whose shape was constant in cysts of all dimensions, were ovoid, blunt and rounded at one end and rather more pointed at the end bearing the

cap, which projected, giving a nipple-like appearance. In size the sporocysts ranged from 6μ to 14μ in the long axis and from $4 \cdot 5\mu$ to 9μ in the short axis.

Almost immediately on the formation of the sporocysts, development of the sporozoites took place. These, when fully formed, were elongated club-shaped bodies, varying from $5 \cdot 75\mu$ to $12 \cdot 5\mu$ in length, by 2μ to $4 \cdot 5\mu$ in breadth at the widest point. They were slightly curved and were arranged within the sporocysts so that the broader end of one overlapped the narrower end of the other. The nucleus was sometimes visible and was centrally placed. It was of the spherical vesicular type with a delicate nuclear membrane and all the chromatin grouped together into a large central karyosome. The cytoplasm of the sporozoites was homogeneous and free from inclusions, but large globular masses of a greenish refractile substance were constantly present, either within or closely overlying the sporozoites. One of these was usually situated at the broad end of each sporozoite, occasionally a second similar body occurred at the narrow end, and rarely, the mass lay between the two sporozoites, partly overlapping each.

A small quantity of residual cytoplasm usually remained in the oöcyst after the formation of the sporoblasts, and a considerable quantity was always present in the sporocysts, irregularly scattered over the sporozoites.

No movement of the sporozoites could be detected at any stage. After being kept for a fortnight without the addition of moisture, the fæces became completely dried up and the oöcysts ruptured, liberating the sporocysts; the sporozoites, however, never became free of the sporocysts. Attempts were made to stain the parasites at various stages of development, but these were unsuccessful owing to the impermeability of the cyst walls.

Discussion.

Although the greater part of the life-cycle of this *Eimeria* is still unknown, it seems probable, from a comparison with the oöcysts of other forms, that it belongs to a species hitherto undescribed.

The chief points of difference between it and other known species lie in the size and shape of the oöcysts and sporocysts and the behaviour of the sporozoites. Unfortunately the descriptions given of many species are scanty, and are unaccompanied by reliable drawings.

The *Eimeria* Parasitic in *Python molurus*.

On two occasions when the fæces of *P. molurus* were examined they proved to contain a few oöcysts, ranging in size from 36μ to 26μ in the long axis by 23μ to 15μ in the short axis. These were morphologically identical with the oöcysts found in the fæces of *P. sebæ* and remained so throughout the course of their development. It is therefore concluded that this *Eimeria* is of the same species as that above described as occurring in *P. sebæ*.

Other Species of Eimeria.

The characteristics of the oöcysts of other species described from reptiles, and the chief points in which they differ from the *Eimeria* found in *P. sebæ* and *P. molurus* may be summarised as follows :—

1.—*E. agamæ* (Laveran & Pettit, 1910).

Found in liver and gall bladder of *Agama colonorum*.
Size of oöcysts 20μ to 25μ, by 11μ to 14μ.
Size of sporocysts 8μ by 4μ.
Size of sporozoites unknown.
The sporozoites become free of the oöcyst and sporocyst.
No cap on the sporocyst described.

2.—*E. cerastes* (Chatton, 1912).

Found in gall bladder of *Cerastes vipera* and *C. cornutus*.
Size of oöcysts 40μ by 20μ.
Size of sporocysts 12μ by 6μ.
Size of sporozoites unknown.
No cap on the sporocyst described or shown in figure.

3.—*E. cystis felleæ* Debaisieux, 1914.

Found in *Tropidonotus natrix*.
Size of oöcysts 30μ to 38μ by 20μ to 25μ.
Size of sporocysts unknown.
Size of sporozoites unknown.
Micropyle not visible.
No cap on the sporocyst described or figured.

4.—*E. crotalæ* Phisalix, 1919.

Found in liver and gall bladder of *Crotalus terrificus*.
Size of oöcysts 32μ by 22μ.
Size of sporocysts $7 \cdot 5\mu$ to 10μ diameter.
Size of sporozoites unknown.
The sporocysts are spherical. The sporozoites become free. No cap on the sporocyst described.

5.—*Eimeria* sp. Phisalix, 1921.

Found in the gall bladder of *Cerastes cornutus*.
Size of oöcysts 25μ by 18μ, or 10μ diameter.
Size of sporocysts unknown.
Size of sporozoites unknown.
The oöcysts are occasionally small and spherical.
No cap on the sporocyst.

6.—*E. zamensis* Phisalix, 1921.

Found in the gall bladder of *Zamensis* sp.
Size of oöcysts 28μ to 30μ by 15μ to 18μ.
Size of sporocysts 10μ diameter.
Size of sporozoites 9μ by 4μ to 5μ.
The sporocysts are spherical, and without a cap.

7.—*Eimeria* sp. Phisalix, 1921.

Found in gall bladder of *Tropidonotus natrix*.
Size of oöcysts 32μ by 20μ.
Size of sporoblasts 7μ to 11μ diameter.
Size of sporozoites 4μ to 11μ by 2μ to 1μ.
The sporoblasts are spherical. The size and shape of the sporocysts is not given.

8.—*E. sinci* (Phisalix, 1923).

Found in bile ducts of *Sincus officinalis*.
Size of oöcysts 32μ by 25μ.
Size of sporocysts 12μ by 9μ.
Size of sporozoites $15 \cdot 6\mu$ by $3 \cdot 6\mu$.

The sporozoites become free in the oöcyst. No cap on the sporocyst described.

From the above summary it will be seen that the *Eimeria* found in *Python sebæ* and *P. molurus* most closely resembles *Coccidium cerastes* Chatton, 1912, from which it differs only slightly in size. From the single text figure given, however, in the description of this species, there appear to be various morphological differences. The micropyle, which is very conspicuous in the *Eimeria* from the Python, is not shown, and there is no trace of the cap on the sporocyst. The shape and arrangement of the sporozoites and sporocystic residuum differ considerably in the two cases, and the nuclei of the sporozoites, centrally placed in the *Eimeria* of the Python, occur at one extremity of the body in *C. cerastes*.

It seems, therefore, most probable that the *Eimeria* found in the fæces of *Python sebæ* and *P. molurus* does not belong to the species *C. cerastes*, but constitutes the type of a new species for which the name *Eimeria pythonis* is suggested. This, however, cannot be conclusively proved until a more detailed description of *C. cerastes* can be obtained. It must here be pointed out, that, according to the rules of nomenclature, *Coccidium cerastes* Chatton, 1912, should in future be known as *Eimeria cerastes* Chatton, 1912.

2.—The Cryptosporidian found in the fæces of *Crotalus confluentus*.

Oöcysts of a cryptosporidian nature were found to form a very heavy infection in the fæces of *Crotalus confluentus* on a single occasion when these fæces were available for examination. As many as six cysts were found in a single field on examination of the fæces in normal saline solution under a one-sixth objective.

The oöcysts were ovoid and ranged in size from 10μ by $10{\cdot}8\mu$ to 11μ by $12{\cdot}5\mu$.

The cyst wall was composed of a single, double-contoured membrane, of a colourless, transparent, hyaline nature, about $0{\cdot}5\mu$ in thickness. No micropyle was visible.

Lying within the cyst, and arranged in no definite order were four elongated sporozoites. These were slightly curved bodies of approximately even thickness throughout their length, but sometimes tapering slightly towards one end. They measured from $6{\cdot}5\mu$ to $7{\cdot}5\mu$ in length,

and from $1 \cdot 5\mu$ to $2 \cdot 25\mu$ in breadth, and were composed of clear, non-granular cytoplasm. The nucleus was not visible in fresh specimens. A small amount of granular residual cytoplasm was also present in the oöcysts.

By exerting slight pressure on smears fixed in Schaudinn's fluid, it was possible to rupture the wall of the oöcysts, and in smears treated thus and stained with Heidenhain's iron hæmatoxylin the nuclei of the sporozoites could be made out. These were oval, of the vesicular type, with eccentric karyosomes and large irregular blocks of chromatin lining the nuclear membrane. They were situated in the broader end of the body. The average size of the nucleus was $1 \cdot 5\mu$ by $1 \cdot 75\mu$, the karyosome being about $0 \cdot 5\mu$ diameter.

Shrinkage took place in the fixative, and the sporozoites appeared rather irregular in shape in stained specimens. The inner surface of the wall of the oöcyst stained intensely but the outer surface remained colourless.

Crumpling of the oöcyst wall took place on dessication, but the sporozoites appeared unchanged and were not liberated either in the dry condition or on the addition of normal saline or distilled water.

Other cryptosporidian parasites, *Cryptosporidium muris* Tyzzer, 1910, and *C. parvum* Tyzzer, 1912, commonly occur in mice and rabbits, and it was thought that, in the present case, the parasite might be present as an organism of passage only. A comparative study of the oöcysts of these forms, however, taken in conjunction with the heaviness of the infection, prove that this is extremely unlikely. The oöcysts of *C. parvum* measure from 4μ to $4 \cdot 5\mu$ by 3μ to $3 \cdot 3\mu$. Those of *C. muris* measure 7μ by 5μ, *i.e.*, little more than half the size of the *Cryptosporidium* found in the rattle snake.

It is therefore concluded that this *Cryptosporidium* belongs to a new species and is parasitic in *Crotalus confluentus*. It is suggested that this species should be given the name of *Cryptosporidium crotali*.

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Plates.

Figs. 1 to 9.—*Eimeria* found in fæces of African Python (×2,000).

1.—Undifferentiated oöcyst as seen in freshly passed fæces.

2.—Oöcyst with contents rounded up into central mass, showing nucleus.

3.—Small oöcyst with rounded up contents.

4.—Oöcyst with contents dividing to form sporoblasts.

5.—Oöcyst in which the formation of sporoblasts is completed, showing oöcystic residuum.

6.—Oöcyst containing sporocysts before the development of sporozoites.

7.—Large oöcyst at last stage of development, showing four dizoic sporocysts.

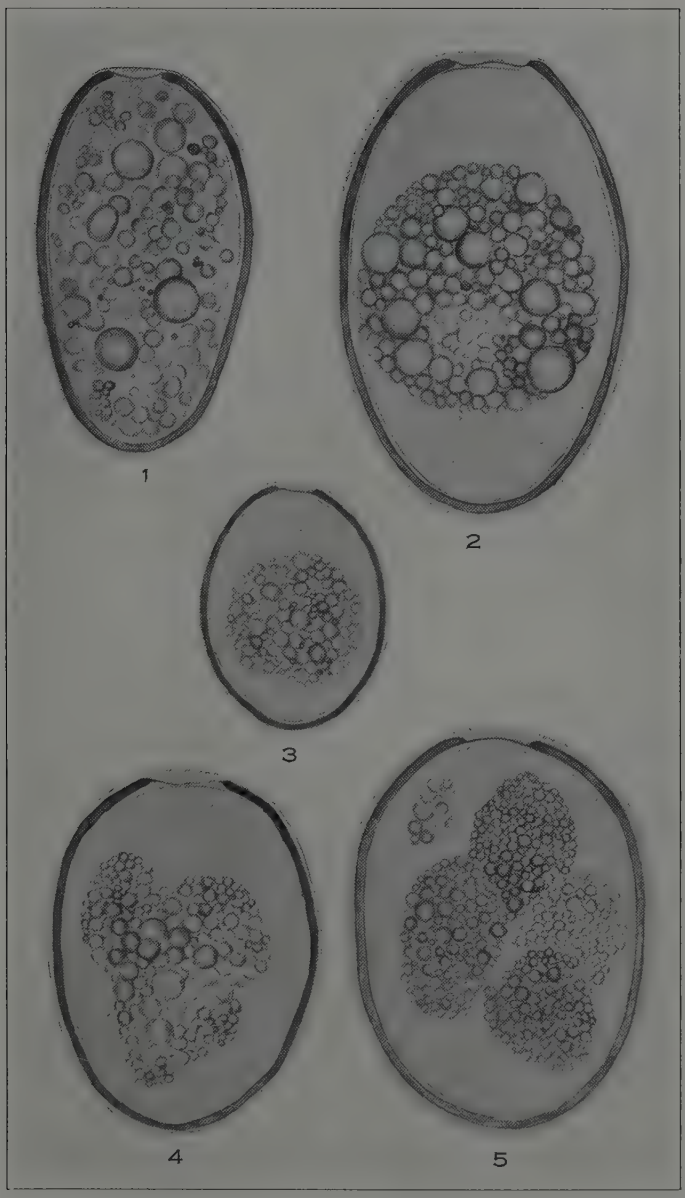
8.—Small form at completion of development.

9.—Single sporocyst from large form showing structure and arrangement of sporozoites.

Figs. 10 to 12.—*Cryptosporidium crotali* (×2,000).

10 and 11.—Fresh oöcysts showing residual material and shape and arrangement of spores.

Fig. 12.—Stained oöcyst showing nuclear structure of the sporozoites.



Figs. 1–5.—*Eimeria pythonis*, sp. n.

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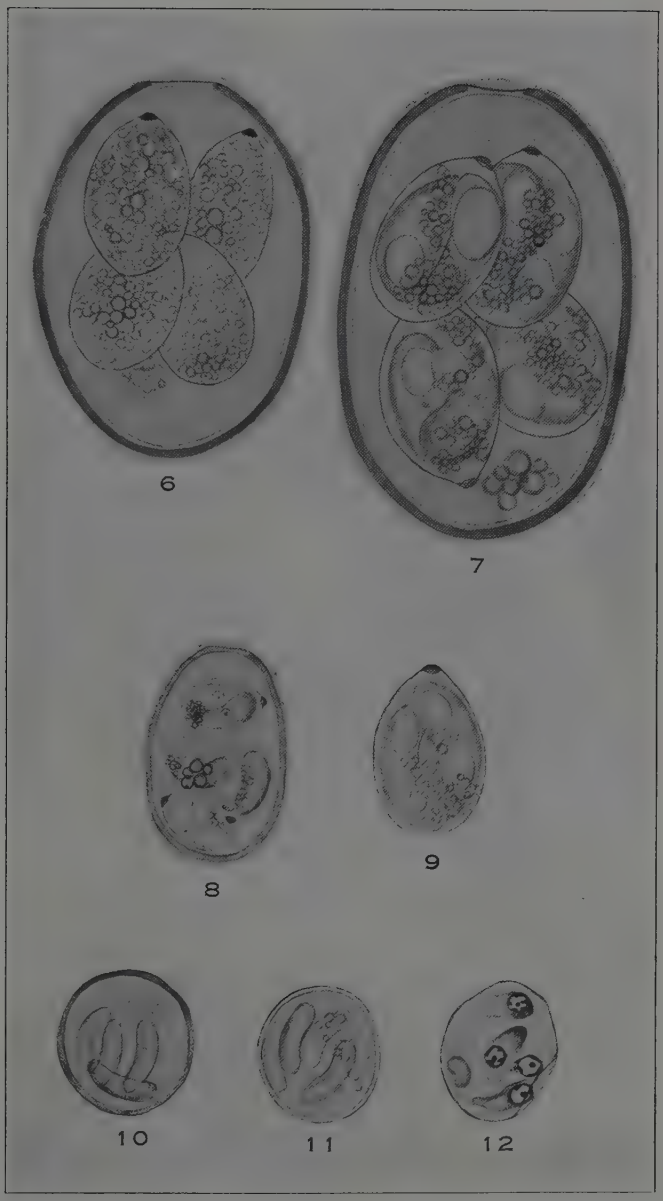

Figs. 6–9.—*Eimeria pythonis.* Figs. 10–12.—*Cryptosporidium crotali*, sp. n.

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Protozoology No. 2, January, 1926, pp. 27–30.

Observations on Amœbæ found in the fæces of certain African Ungulates.

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DURING a series of examinations of the fæces of ruminants belonging to the Zoological Society of London amœbæ were found to be constantly present in freshly passed material from certain African Ungulates.

From a study of these forms both in the living condition and in stained preparations, it appeared that two distinct organisms were represented. The first of these was present both in the free form and encysted, in the fæces of a Sable Antelope (*Hippotragus niger*) from South Africa, and a pair of Common Waterbuck (*Cobus ellipsiprymnus*) from East Africa. The second form was found in the fæces of an Eland (*Oreas canna*).

The amœba in the Sable Antelope formed a very heavy infection. In the Waterbuck the infection was lighter but the parasites were still numerous. Cysts and free vegetative forms were present on each occasion when examinations were made, but the free forms were always comparatively scanty. The parasite of the Eland was represented by cysts only, and was present on all six occasions when the fæces were examined. The cysts, however, varied greatly in number from day to day, being scanty in some specimens and very numerous in others.

THE AMŒBA IN THE SABLE ANTELOPE AND WATERBUCK

When freshly passed fæces were examined in normal saline solution the free amœbic forms were seen to be motile. The pseudopodia were broad and bladelike, and were composed almost entirely of ectoplasm,

which formed a narrow but distinct zone surrounding the endoplasm of the body. The endoplasm was finely granular and slightly vacuolated. The nucleus could be distinguished as a large, clear, rounded area, with, apparently, a very large central karyosome. Movements ceased and the amœbæ rounded up within three or four hours after the fæces were passed. The rounded up forms varied from 8μ to 12μ in diameter.

Smears stained with Heidenhain's Iron Hæmatoxylin showed a clear differentiation between ecto- and endoplasm. A few food particles, usually of a bacterial nature, were present in the endoplasm. No engested red blood cells were found. The nucleus was large and spherical, with a fine but distinct membrane lined by chromatin masses. These varied in size and number, but were constantly evenly spaced. The remainder of the nuclear structure varied considerably both in the free forms and in the cysts. In every case a deep-staining karyosome was present, usually central, but sometimes slightly eccentrically placed. In the majority of the free forms and many of the cysts a number of deep-staining granules were present closely surrounding and sometimes almost masking the karyosome. These granules were often somewhat indeterminate in form, and seemed to merge together to form a definite darkly stained circular zone. The karyosome in almost all cases could be distinguished as a more intensely stained central dot. Other forms, more particularly the cysts, showed the karyosome surrounded by a clear area or halo. The structure of the intermediate zone between this clear space and the nuclear membrane again varied. Frequently the clear area was bounded by an indeterminate granular mass, in which, and occasionally outside which, a few large distinct granules could be made out. In other cases ring-like structures were present formed either of large granules, few in number, or more numerous small, deeply stained granules. The size of the nuclei varied in the free forms from 4μ to 6μ diameter, and in the cysts from 2·25μ to 4μ diameter. The karyosome varied from 0·25μ to 1μ in diameter. The cysts were usually spherical, occasionally ovoid, and were all uninucleate. The cytoplasm was slightly granular and contained numerous vacuoles. In Gramm's Iodine the contents of these vacuoles stained a deep brown, indicating that they contained glycogen or some allied substance. Scattered throughout the cytoplasm, and often arranged around the vacuoles, were deeply staining masses apparently of a chromatoid nature. These chromatoid bodies

varied greatly in shape and size ; many were rod-shaped, either straight or curved, with rounded ends, others were irregular in outline, and others again were rounded. In the Sable Antelope the cysts measured from 7μ to 12μ, while in the Waterbuck they tended to be slightly larger, varying from 10μ to 15μ in diameter.

The Amœba in the Fæces of the Eland.

As stated above, no free forms were found, but cysts were usually numerous. They varied from 5μ to 12μ in diameter, and were frequently slightly irregular in outline. The cyst wall measured about 0·5μ in thickness. A single large glycogen vacuole was usually present. The cytoplasm was faintly granular, and contained chromatoid bodies of two types. These were thick rod-shaped forms with bluntly rounded ends and small circular bodies. Both types were usually present in each cyst, but in a few cases only the small rounded forms were present. The nuclei were spherical and vesicular. The nuclear membrane was very fine and lined with dots of chromatin. Occasionally a cyst was found in which these chromatin dots tended to be aggregated towards one pole, where they formed a crescent-shaped mass. The karyosome was well marked and usually centrally placed. There was no intermediary chromatin between the karyosome and the nuclear membrane, but fine linin threads could usually be made out extending radially between the two. The nuclei in this case varied in size from 1·5μ to 3·5μ. As in the cases described above, only uninuclear cysts were found.

Discussion.

Owing to the disparity in nuclear structure between these forms it is concluded that they represent two separate species of the genus *Entamœba*.

The form described from the Sable Antelope and Waterbuck shows many morphological resemblances to *Entamœba ovis* Swellengrebel, 1914. The nuclear structure of *E. ovis* varies in much the same way as is described above for the amœba in the Sable Antelope and Waterbuck ; while the cysts, which are all uninucleate, contain rod-shaped or irregular chromatoid bodies which are frequently arranged round the periphery of a large vacuole. The sizes given for this amœba are 12μ by 11μ to 14μ by 12μ for the free form, and 8μ by 8μ for the cysts. Thus the free forms are slightly larger than any forms found in the present case, while

the cysts are slightly smaller than the average size found in the Sable Antelope. There appears, however, to be little doubt that the amœba above described is *Entamœba ovis* occurring in a new host.

With regard to the amœbic cysts found in the Eland, no record can be found of any similar form occurring in ruminants. The general morphology of the parasite most closely resembles that of *Entamœba histolytica*, but no binuclear or quadrinuclear cysts were found. It appears, therefore, that this form probably belongs to some hitherto undescribed species of the Genus *Entamœba*.

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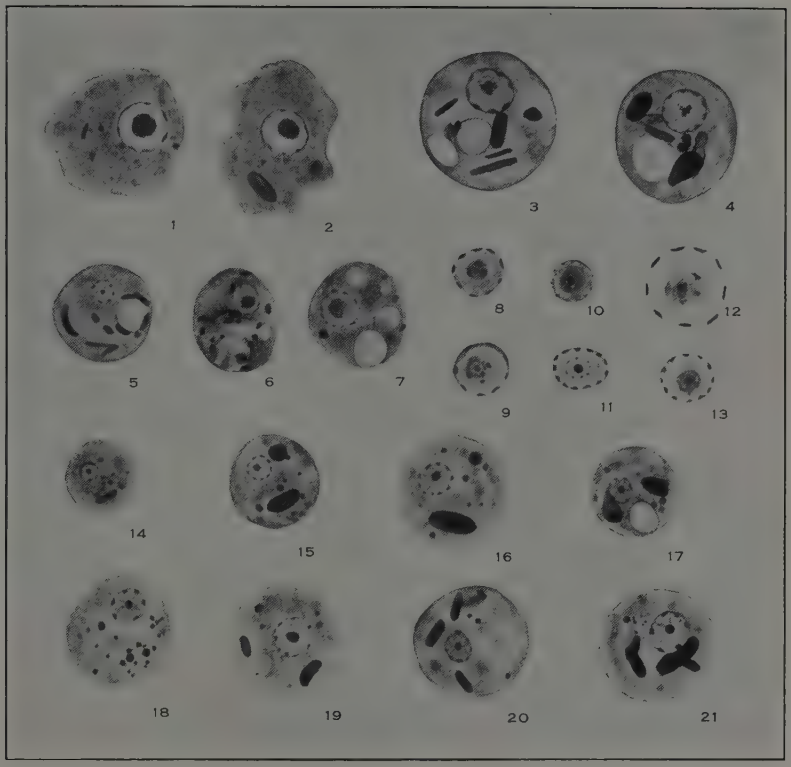

AMŒBÆ from African Ungulates

Figs. 1 and 2.—Free vegetative forms from the fæces of the Sable Antelope. ×2000.

Figs. 3 to 7.—Cysts from Waterbuck and Antelope showing nuclear structure chromatoid bodies and glycogen vacuoles. ×2000.

Figs. 8 to 13.—Various types of nuclear structure found in amœba of Antelope. ×2000.

Figs. 14 to 21.—Cysts of amœba found in fæces of Eland showing nuclear structure, chromatoid bodies and glycogen vacuoles. ×2000.

To face p. 30)

Protozoology No. 2, January, 1926, pp. 31–46.

Some Sporozoan Parasites found in the intestinal wall of Bennett's Wallaby (*Macropus bennetti*).

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Introduction.

At the Laboratory Meeting of the Royal Society of Tropical Medicine and Hygiene in March, 1925, Drs. C. M. Wenyon and H. H. Scott exhibited sections of the small intestine of a Bennett's Wallaby (*Macropus bennetti*), which had died in the Zoological Gardens of London. The sections contained two organisms which showed a close morphological resemblance to *Ileocystis macropodis* and *Lymphocystis macropodis* respectively, described by Gilruth and Bull from the intestine of a Kangaroo (*Macropus* sp.). In addition to these parasites, vacuolic spaces containing spore-like bodies were present in the muscle layers, and the superficial epithelium was heavily parasitised with various stages in the life cycle of a coccidian. As the fæces of other wallabies which had lived in association with the infected individual, were found, on examination, to contain oöcysts of an *Eimeria*, it was concluded that these oöcysts belonged to the same species as the developmental forms already seen. The name *Eimeria macropodis* was accordingly given to this parasite, the specific characters being based on the morphology of the oöcysts. A report of this demonstration, accompanied by a brief account of the various organisms, was published in the Transactions of the Royal Society of Tropical Medicine and Hygiene, vol. xix., No. 1, 1925.

The purpose of the present paper is, therefore, to give a more detailed description of the morphology of these organisms, and to provide figures which may be of use for comparative purposes.

I wish here to express my thanks to Dr. Scott, who has very kindly placed the material at my disposal; and to Dr. J. G. Thomson for his valuable criticism and advice.

Methods.

The material from which the sections were cut was fixed in Bouin's fluid. Various staining methods were used, the best results being obtained with Heidenhain's Iron Hæmatoxylin. Unfortunately no smears were available, so that the wet-fixation characters of the nuclei were lost. In using the same technique with a similar sporozoan parasite, *Globidium gilruthi* (*Gastrocystis gilruthi*), when fresh material was available for comparison, considerable shrinkage was found to take place. It seems, therefore, necessary to emphasise the fact that, with the exception of the oöcysts of the *Eimeria*, all the organisms described in this paper have been studied only in the fixed and stained condition.

Associated Lesions.

In a small limited area the surface epithelium of the mucous membrane was very heavily parasitised with developmental forms of a coccidian. Almost every cell was doubly infected, and, where mature schizonts and gametocytes occurred in groups the host cells were completely obliterated. Another portion of the tissue showed an acute inflammatory area where the epithelium had been completely destroyed, and numerous petechial hæmorrhages were visible. This denudation of the mucous layer was accompanied by the formation of a pseudo-membrane and a marked thickening of the subjacent tissues. Evidences of a general inflammatory condition were present in the hypertrophied sub-mucosa including a general rarefying œdema and a heavy infiltration of small cells, a high percentage of which were polymorphonuclear leucocytes. It was in this region that the spore-forming organisms resembling *Ileocystis* and *Lymphocystis* were most common.

The circular muscle layer, although locally parasitised, appeared to be unchanged.

The Parasite resembling *Ileocystis macropodis*

This organism was represented by large oval forms, apparently mature, containing numerous spore-like bodies and measuring from 40μ to 70μ in length. Smaller forms were present in which spore formation had not taken place, and, by a careful search of numerous sections it was found that a fairly complete series of developmental stages could be traced, illustrating the life history of the parasite from a small uninuclear stage, measuring only 8μ in diameter, up to the large forms mentioned above.

The younger stages of this parasite were found both in the epithelial layer in those areas where the coccidian infection was less intense or absent, and, more frequently, in the thickened region of the sub-mucosa. In this latter position they tended to occur in groups, and were often closely adjacent to the more mature stages. Multinuclear and mature forms were found only in the sub-mucosa where they were either isolated or aggregated together in clusters of three or four.

The morphological structure of the actual parasite in the youngest forms was comparatively simple, and remained unchanged until a diameter of about 15μ was attained.

The smallest stage which was found consisted of an irregularly rounded uninuclear body of only 4·5μ diameter, embedded in the cytoplasm of a cell of the sub-mucosa. The nucelus of the host-cell was slightly displaced and distorted into a semilunar shape, into the concavity of which the body of the parasite fitted. The cytoplasm of the host cell remained unchanged except for a very thin, membrane-like zone around the parasite, which stained a faint blue in Erlich's Acid Hæmatoxylin, and a deeper grey shade than the remainder of the cytoplasm in Heidenhain's Iron Hæmatoxylin. The diameter of the whole body, including the host cell, varied between 8μ and 8·5μ.

The cytoplasm of the parasite was faintly staining and very markedly vacuolic, giving the nucelus the appearance of being suspended in the centre of the body by a few cytoplasmic threads. The nuclear structure was very characteristic and was uniform, except for the apparent loss of the nuclear membrane, throughout all the developmental stages up to the actual formation of the spores, where it could no longer be traced. At this stage the nucelus, which was roughly circular in outline, measured only 1·5μ in diameter. The nuclear membrane was distinct, and the chromatin was massed together to form a single large, somewhat eccentrically placed karyosome. The intermediate zone between the karyosome and the nuclear membrane stained faintly with chromatin stains and had a faintly granular appearance.

From this small stage onwards, the remainder of the endogenous cycle, as interpreted from the forms present in the available material, appeared to be as follows :—

Progressive growth of the parasite takes place, accompanied by stretching of the host cell. The morphology of the parasite remains unchanged, and the ratio between the diameter of the nucelus and the diameter of the body is constant until the body attains about three times its original size, *i.e.*, about 15μ diameter. The host cell then forms a membrane or envelope around the parasite of about 2μ average thickness, except at one pole, where it is thickened owing to the presence of the nucleus, which forms a crescentic band closely applied to the parasite.

With further growth the organism loses its spherical outline and becomes ovoid. With this change of shape a morphological change also becomes evident. The cytoplasm, which in the rounded stages is faintly staining and sparse, apparently increases in mass, and the majority of it becomes aggregated around the nucleus, where it forms a deeply-staining network of cytoplasmic threads. At the same time, the outer margin of the envelope tends to lose its regular definite contour, and short ill-defined processes project from it at irregular intervals. At the same time, the substance of the envelope shows a faint striation, indicating a fibrillar structure, and it may, especially in the thickened region around the nucleus, become more or less coarsely vacuolated.

Following on the formation of the envesting mass of cytoplasm, the nucleus divides, forming two daughter-nuclei, smaller in size but otherwise identical with the original.

Since the mature stage varies so greatly in size, it is not possible to decide whether growth continues throughout the whole period of nuclear division and spore formation, but as no mature stages of less than 40μ in length were found, it seems probable that at least the first nuclear divisions take place during the period of growth.

Further nuclear divisions occur and the central mass of cytoplasm continues to increase in volume and appears to be suspended in the central cavity by strands of the original faintly staining cytoplasm, which still persists, forming a lining to the inner margin of the envelope. At the stage when about twenty nuclei are present they tend to be arranged round the periphery of the central cytoplasm. Later, however, when further proliferation has taken place, they appear to lie in linear series, as though arranged upon a curved, slightly branching thread disposed throughout the cytoplasm. A progressive decrease in the size of the

nuclei is noticeable as division continues. At the stage where from 30 to 40 nuclei are present in a single meridional plane the average diameter is only 0·75μ, and although the karyosome remains clearly visible the nuclear membrane cannot be distinguished and appears to have been lost.

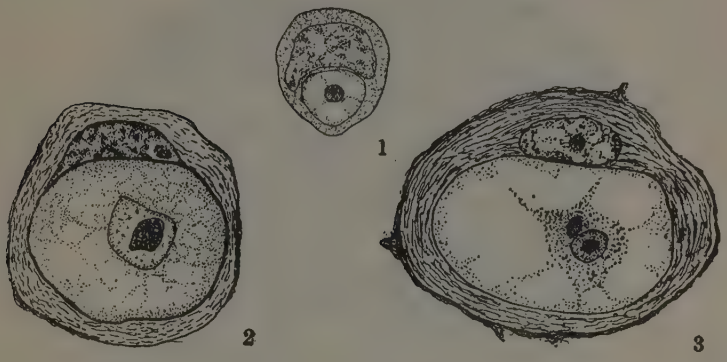


Fig. 1.—Youngest stage of large spore-forming parasite (*Globidium mucosa* ?) showing slight distortion of host cell, vacuolated cytoplasm and nuclear structure of parasite. ×2,000.

Fig. 2.—Older uninucleate stage showing further distortion of host cell and fibrous appearance of its cytoplasm. ×2,000.

Fig. 3.—Oval binuclear stage, showing outgrowths from the envelope cell, and coarsely granular cytoplasm surrounding the nuclei. ×1,500.

Unfortunately no intermediate forms could be found between the stage shown in Fig. v., where the granular cytoplasm does not completely fill the envelope, and the mature stage where the envelope is completely filled with spores. Nothing is known, therefore, regarding the final arrangement of the nuclei before the actual process of spore formation.

In the mature stage the cyst wall is thinned to an average width of 1·5μ except in the region containing the nucleus. The whole of the central cavity is filled with loosely packed, residual cytoplasm, in which are scattered innumerable spores, apparently lacking any definite arrangement. The morphology of the spores could not be very accurately ascertained from the sections owing to their minute size. They appeared to

be club-shaped, slightly curved bodies with one end rounded, the other tapering to a point. In the round end the nucleus was visible as a deeply staining dot. The size of the spores was about 3μ to 5μ in length by about 2μ to 2·5μ in width at the rounded end.

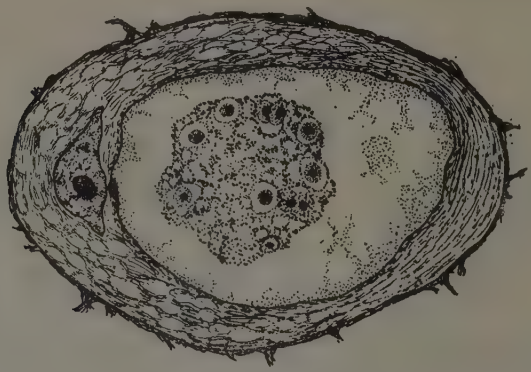


Fig. 4.—Multinuclear stage, showing vacuolisation of envelope and large central mass of cytoplasm with peripherally arranged nuclei. ×1,500.

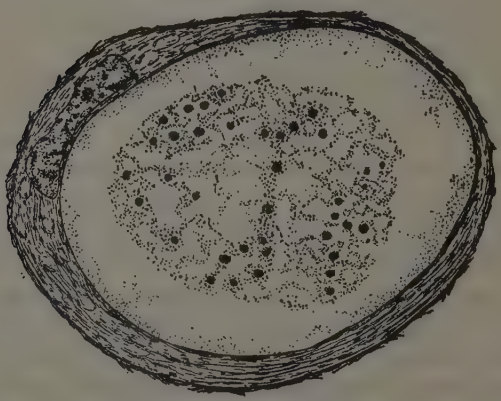


Fig. 5.—Later stage in nuclear proliferation showing linear arrangement of nuclei. ×1,500.

Affinities.

Morphologically this parasite, in its more mature stages, almost exactly resembles the organism *Ileocystis macropodis* described and named by Gilruth and Bull. There are, however, several minor points of difference which should be noted. These differences lie both in the structure of the host cell or envelope, and in the structure of the body of the parasite.

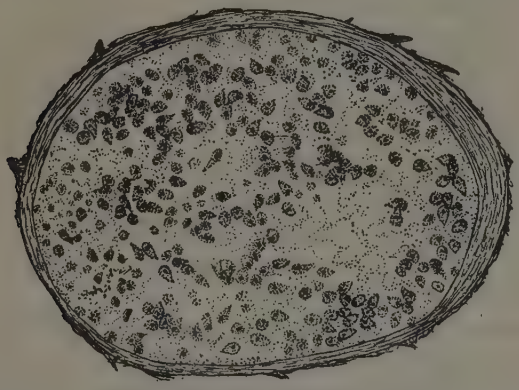


Fig. 6.—Mature stage containing spores scattered throughout residual cytoplasm. × 1,500.

In *Ileocystis macropodis* the envelope was provided with an outer fringe-like covering of delicate processes, which formed a definite zone of about 8μ in depth. The bases of these processes could be traced into the outer layer of the envelope, giving to the latter the appearance of a delicate transverse striation. Where the bodies were closely approximated this fringe-like outgrowth appeared only at the free borders where the envelopes abutted upon the normal tissues of the host. In the parasite found in Bennett's Wallaby the outer margins of the larger cysts were irregular and bore short outgrowths, but these were too indefinite in arrangement and irregular in form to be described as a fringe ; moreover, they measured not more than 4μ in length. The vacuolisation and longitudinal striation seen in the envelope of the parasite described above were neither mentioned nor figured as appearing in *Ileocystis macropodis*.

With regard to the body of the actual parasite, Gilruth and Bull described this as consisting of a homogeneous endoplasm within which is situated one blastophore or schizont. From this description and from a study of the photomicrographs and figures in the original paper, it appears that the space lying between the envelope and central mass of nuclei and cytoplasm was filled by some substance of a homogeneous structure instead of appearing as a hollow vacuolic space as in the parasite found in Bennett's Wallaby.

It seems possible, in fact probable, that these differences are merely artifacts due to different methods and technique adopted in preparing the sections, and that the two parasites are in reality identical.

The Organism parasitic in the Lymphocytes.

This was recognisable only in the thickened region of the sub-mucosa. It formed primarily an infection of the lymphocytes, but many spores were found lying free in the intercellular spaces, this being probably due to the destruction of the host cells.

The invaded cells were greatly hypertrophied. The nucleus was distorted to a roughly crescentric shape and displaced to one edge of the cell, while the cytoplasm formed a fine, delicate, membrane bounding a large central vacuole which contained the parasite. Only one stage of development was represented in the sections, namely, the mature stage with fully developed spores. These were club-shaped and slightly curved with one end bluntly rounded, the other slightly more pointed. The cytoplasm was homogeneous and free from granules. They measured about 6μ in length by 2μ to $2{\cdot}5\mu$ in breadth. The nucleus, which was situated in the thicker end of the body, and was usually marginally placed, was oval in outline and rich in chromatin. These spores varied in number within a single lymphocyte from twelve, which was the usual number, up to about twenty. They were arranged without any apparent order and no residual cytoplasm was ever observed.

Frequently, where two or more lymphocytes were closely adjacent, the limiting membranes formed by their cytoplasm had become ruptured so that the contained spores formed a single mass surrounded by the remains of their original host cells. This often gave the erroneous appearance of a single very large group of spores envested by a common envelope.

The free spores lying in the intercellular spaces were either grouped together or completely isolated. The isolated spores commonly occurred at some considerable distance from one another, and many were found closely opposed to normal lymphocytes; it was, however, impossible to decide whether these lay within, or merely upon, the lymph cells.

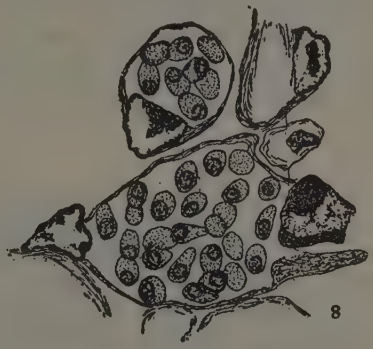


Fig. 7.—Lymphocyte from sub-mucosa containing spores of parasite resembling *Lymphocystis macropodis*. (×2,000).

Fig. 8.—Portion of submucosa showing spores within lymphocyte and large mass of spores surrounded by broken down remains of original host cells (*Lymphocystis macropodis* ?). ×2,000.

This organism is identical both in habitat and distribution with *Lymphocystis macropodis* (Gilruth and Bull, 1912). Morphologically, however, the spores of *L. macropodis* differed from those described above in that those found free in the intestinal contents were described as containing deeply staining granules in their cytoplasm. Furthermore, a single lymphocyte was figured containing 62 spores, whereas 20 was the maximum number found in the present case. In size the spores of *L. macropodis* varied from 4·3μ to 5·5μ by 2·1μ to 2·5μ in the sectioned material, and from 10·7μ to 12·4μ by 2·78μ to 4·25μ in the fresh state as found in the intestinal contents. The spores found in the sectioned material from Bennett's Wallaby were intermediate in size between these two extremes, and no material was available for making smears of free spores. It seems, therefore, feasible that the morphological differences are again due to the technique and that *Lymphocystis macropodis* is identical with the parasite described above.

The Spore-like Bodies of the Muscle Layer.

Many fibres of the muscle layer were distended by the presence of groups of spores which lay within large vacuolic spaces in their cytoplasm. These spores were identical with the ones already described as occurring in the lymphocytes and intercellular spaces of the sub-mucosa. They varied in number from three or four up to thirty or forty within each group. No developing forms were found, and there was no cytoplasmic residuum within the vacuoles. The cytoplasm of the host cells was reduced to a thin limiting membrane but retained its normal staining reactions.

Fig. 9.—Portion of circular muscle showing three groups of spores in muscle fibres. × 2,000.

The nature of these spores could not be determined. Their morphological identity with the spores in the lymphocytes suggests that they might be present as a result of the migration of freed spores of the former organism. Against this view, however, is their constant grouping together. Further, it seems possible that the parasites in the muscle-layer represent another phase in the life cycle of either of the forms in the submucosa. Lastly there remains the possibility that they represent an infection of some totally different organism, as, for example, a *Sarcocystis*.

The Coccidian Parasite.

This was limited to a small area of the epithelium where it formed a very intense infection.

Undifferentiated trophozoites were very numerous. The smallest of these were circular in outline and of about 2·5μ diameter. With increase in size the outline became progressively ovoid, with the long axis parallel

to that of the host cell. Undifferentiated trophozoites reached a maximum size of 10μ by 8·5μ. The cytoplasm of these early forms was constantly homogeneous and the nucelus was either central or eccentrically placed. Nuclear division in the formation of the schizont commenced before the completion of growth. The daughter nuclei arranged themselves around the periphery of the body. Merozoites were formed in the usual way, and varied in number from 8 to 32. They measured about 8μ in length by 2μ to 2·5μ in breadth, and were composed of finely granular cytoplasm, with the nuclei situated in the thicker end of the club-shaped body. A small amount of residual cytoplasm was usually present.

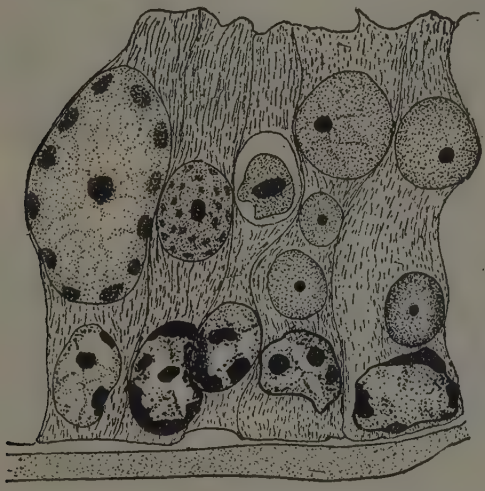


Fig. 10.—Epithelial cells of mucous membrane invaded by developing forms of *Eimeria macropodis*, trophozoites and two stages in development of macrogametocyte. × 2,000.

Male gametocytes were fairly numerous. Immature forms were distinguishable from developing schizonts by the greater number and more deeply staining character of their nuclei. When fully mature, the microgametocytes measured about 32μ in length by 22μ in breadth. The microgametes were very numerous and of the usual type. They measured 3·5μ in length by 0·75μ in breadth at the wider end.

Female gametocytes were present at all stages of development, young forms, however, being by far the most numerous. Early stages were distinguishable by the presence of large deeply staining granular masses scattered throughout the cytoplasm. With increase in size of the gametocytes these granules became more definite and arranged themselves at regular intervals in the most superficial layer of the cytoplasm. They showed great variation in both size and number at all stages in the development of the gametocyte. Two examples were found in which the wall of the oöcyst was in process of formation. In the first of these the

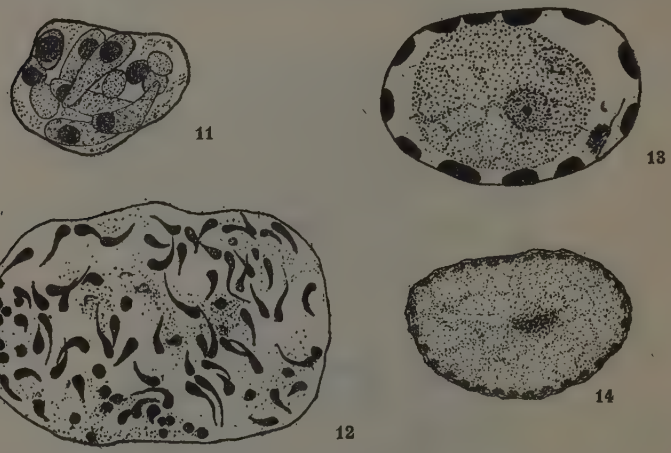
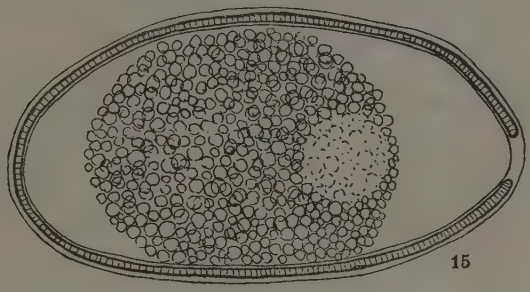
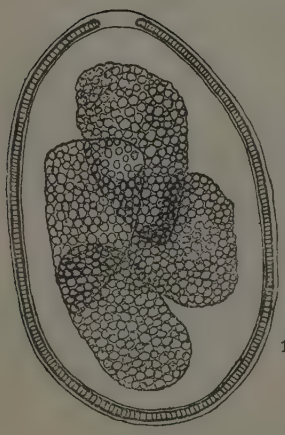


Fig. 11.—Schizont of *Eimeria macropodis* with twelve merozoites. ×2,000.
Fig. 12.—Microgametocyte of *Eimeria macropodis*. ×2,000.
Figs. 13 and 14.—Young oöcysts of *E. macropodis* from epithelium, showing development of cyst wall and characteristic shape. ×2,000.

parasite completely filled the cyst and numerous small granular masses which were embedded in the superficial cytoplasm were closely applied to the deep-staining, much crinkled membrane forming the oöcyst wall. In the second example the cytoplasmic contents had shrunk away to form a central ovoid mass leaving the large intensely staining marginal blocks adhering to the outer walls. Both of these oöcysts showed the slight asymmetry which characterised those found in the fæces of other wallabies.

Examination of the fæces showed that the infection in other individuals was heavy, many oöcysts being present. They were ovoid, and tended to be asymmetrical, being slightly flattened on one side, convex on the other. They varied in length from 22μ to 34μ, and in breadth from 10μ to 17μ. Large oöcysts of average size, 30μ by 15μ, were most numerous, and small forms of 22μ to 23μ by 10μ to 11μ were also common,





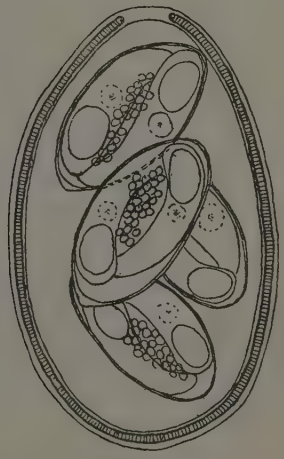


Fig. 15.—Undifferentiated oöcyst of *E. macropodis*.
Fig. 16.—Oöcyst showing four irregularly shaped sporoblasts.
Fig. 17.—Fully developed oöcyst showing four di-zoic sporocysts. (×2,000).

comparatively few intermediate forms being found. The wall was composed of the usual three layers. The middle layer was about 1μ in

thickness, and had a brownish tint. The micropyle was clearly visible as a gap of about 3μ wide in the thick middle wall.

The contents of the freshly passed oöcysts varied. In the majority the protoplasmic contents had shrunk away from the cyst wall to form a central spherical mass. The cytoplasm of this mass was finely granular and the nucleus was visible as a clear rounded area of from 5μ to 7μ diameter. A few of the oöcysts showed their contents divided up into four very irregularly shaped sporoblasts in which the nuclei were again visible.

Forty-eight hours after the fæces were passed the sporocysts were fully developed. These were ovoid with one end slightly pointed. At this pointed end a thickening occurred in the cyst wall forming a refractile knob. The dimensions of the sporocysts varied proportionately with those of the containing oöcysts from 7μ to 11μ in length, and from 6μ to 8μ in breadth. There was no oöcystic residuum. The sporozoites were of the usual type, club-shaped and slightly curved. They measured slightly less in length than the containing sporocysts, and were of about 2μ to 3μ in breadth at the thick end. The nuclei of the sporozoites were more or less centrally placed and were of the spherical vesicular type. Lying usually in the thick end, sometimes in the narrower end of the sporozoites was a clear refractile globular mass. A varying amount of residual cytoplasm was present in each sporocyst lying between, and partially extending over the sporozoites. This was constantly present but, as stated above, there was never any trace of residual material in the oöcyst.

The name *Eimeria macropodis* was given to this coccidian by Wenyon and Scott, the diagnostic specific characters being as follows :—The size and frequent asymmetry of the oöcysts, the size of the sporocysts and the presence of a thickened refractile knob on their more pointed ends ; the absence of any oöcystic residuum.

General Discussion and Nomenclature.

There seems little doubt that the first two parasites described in this paper are, if not identical, at least very closely allied with *Ileocystis macropodis* and *Lymphocystis macropodis* respectively. A comparison of these forms with similar spore producing organisms, more especially

of those found in the walls of the alimentary tract of other mammals, shows that many morphological characters are common to them all.

Nöller, in Prowazek's " Handbuch der Pathogenen Protozoen," places all these parasites under a common generic title, namely *Globidium*, of which the type species is *G. leuckarti*, Flesch, 1883, a parasite found in the intestine of the horse. This mode of nomenclature seems advisable in the present state of our knowledge, but it must be considered as being strictly provisional in view of the strong morphological divergence found between some of the species and also from the fact that the life cycle and mode of infection remains unknown. Thus *Gastrocystis smithi*, described by Railliet, 1919, should also be included provisionally in the genus *Globidium*.

The species comprised, therefore, under the genus *Globidium* are as follows :—

(1) Type species *Globidium leuckarti* Flesch, 1883.
(2) *Globidium mucosa* (Blanchard, 1885), Nöller, 1920. Syn. *Ileocystis macropodis* Gilruth and Bull, 1912, and *Haplogastrocystis* nov. gen. Nöller, 1920, Chatton, 1912.
(3) *Globidium gilruthi* (Chatton, 1910) Nöller, 1920. Syn. *Gastrocystis gilruthi*, Chatton, 1910.
(4) *Globidium macropodis* (Gilruth and Bull, 1912) Nöller, 1920. Syn. *Sarcocystis macropodis* Gilruth and Bull, 1912.
(5) *Globidium* sp. (Gilruth and Bull, 1912) Nöller, 1920. Syn. *Lymphocystis macropodis* Gilruth and Bull, 1912.
(6) *Globidium wombati* (Gilruth and Bull, 1912) Nöller, 1920. Syn. *Ileocystis wombati* Gilruth and Bull, 1912.
(7) *Globidium besnoiti* (Marotel, 1912) Nöller, 1920. Syn. *Gastrocystis besnoiti*, Marotel 1912, and *Besnoitia* nov. gen. Franco and Borges, 1916.
(8) *Globidium smithi* (Railliet, 1919) Triffitt, 1925. Syn. *Gastrocystis smithi* Railliet, 1919.
(9) *Globidium tatusi* Cunha and Torres, 1925.

The specific name of *Lymphocystis macropodis* falls as a homonym on being placed in the genus *Globidium* (5). A new name is not suggested at present.

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On the Presence of Flagellates in the Intestine of the Nematode *Diplogaster longicauda*.

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Introduction.

About half-a-dozen decaying Narcissus bulbs attacked by *Tylenchus dipsaci*, part of a large quantity of diseased bulbs planted on an experimental plot at the Institute's Field Station, Winches Farm, had been placed in a wide-mouthed glass jar, on the floor of which was a layer of wet cotton wool so as to render the jar a moist chamber. The bulbs were raised above the level of the wool on a piece of wire netting, so that they did not stand in water. After several weeks they were examined, and in teasing out some of the moist brown decayed material from one of the bulbs, large numbers of the common free-living nematode *Diplogaster longicauda* were found.

Whilst studying the structure of the worms, one of us (T.G.) found an adult female specimen in which actively moving bodies were seen within the lumen of the intestine. The cover-slip was sealed to the slide and further examination was made under the oil-immersion lens, when the motile bodies were found to be actively swimming flagellates, each one provided with a single anterior flagellum.

They were present in large numbers, probably well over a hundred, though a count was impossible owing to their great motility. Most of them were seen at the anterior end of the intestine, but a few were also found at the posterior end and one was seen to be shot out through the anus into the surrounding liquid.

A camera lucida drawing of the posterior end of the intestine was made with one or two flagellates in situ, and some measurements were also taken. The cover-slip was then carefully lifted and the infected worm was transferred to a clean cover-slip by means of a capillary pipette. Another cover-slip was placed on this and the nematode was crushed between the two so as to give two film preparations, which were immediately fixed in Carnoy's fluid. Subsequently, they were stained with Ehrlich's Hæmatoxylin and counterstained with Biebrich scarlet, with the result that well-stained flagellates were found showing a single flagellum and a fusiform body with kinetoplast and trophonucleus.

Further examinations of the decayed bulb material showed that large numbers of *D. longicauda*, male, female and immature larvæ, were infected with the flagellates, some more heavily than others, and from this time the investigation of the organisms was undertaken jointly by the two authors.

It may be noted here that the flagellates were first seen on December 20th, 1926, and the original rotting bulb material mixed with water in which the nematodes were found has remained in the Petri dishes from that date to the present time, three months later. During this period there has been a constant supply of infected worms furnishing the material on which the observations recorded in this paper have been carried out.

Historical.

There are four records of flagellates occurring in the intestine of nematodes previous to the present one:

(1) Bütschli (1878) discovered in the intestine of the free-living nematode, *Trilobus gracilis*, numbers of a small spindle-shaped flagellate about 11μ in length, each having a flagellum about twice the length of the body. His figures show an organism very similar in appearance to the flagellates described in the present paper, a point which is discussed at greater length on p. 58.

(2) Chatton (1924) describes the finding of rather large leptomonad flagellates in the gut of an undetermined marine nematode which he had dissected alive. The worm was a large one, about 1 cm. long, and the flagellates also were of considerable size, measuring 20μ in length with a flagellum of the same length.

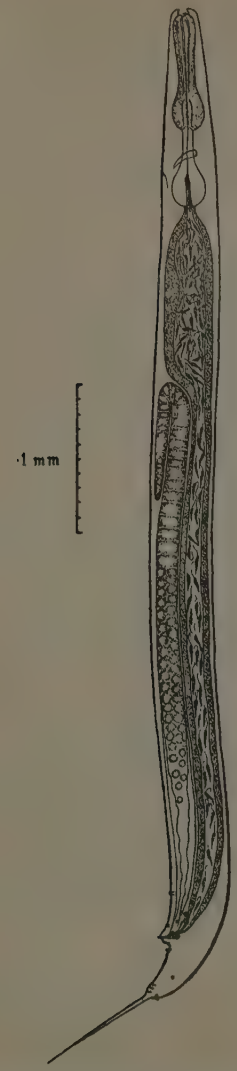

Fig. 1.—*Diplogaster longicauda*, male, showing main features in the anatomy of the worm and numerous flagellates within the intestine.

(3) Whilst examining living examples of the nematode *Viannella viscaciæ*, a parasite from the small intestine of the South American rodent, *Viscacia viscacia*, one of us (Goodey (1925)) found large numbers of the flagellate *Giardia* actively moving in the intestine. These organisms were studied by Dr. J. G. Thomson of the Protozoology Department, London School of Hygiene and Tropical Medicine, who published a paper on them in the first number of this JOURNAL—Thomson (1925).

(4) In dealing with the discovery of the *Giardia* in *Viannella viscaciæ* mention was made of the fact that the writer, Goodey, had on one previous occasion seen a flagellate swimming in the intestine of a nematode obtained from rotting celery. This worm was also a female of *Diplogaster longicauda*, but beyond noting the presence of the flagellate no further observations were made on the subject at that time.

OBSERVATIONS ON LIVING MATERIAL.

On many occasions since first finding the flagellates in the Diplogaster from the decaying bulb mixture, they have been studied for long periods under high magnification in the living worms. This has been done by collecting a number of the nematodes by means of a capillary pipette under the low power of the dissecting microscope. The worms are then transferred to a small drop of liquid on a slide and covered with a No. 1 cover-slip, care being taken that the film is sufficiently thin to prevent the worms from swimming too freely about. The edges of the cover slip are sealed to the slide by painting round with the hot wick of a candle. Individual nematodes can now be examined easily, and under the two-thirds objective it can be determined whether a given worm is infected or not. By bringing an infected one under the oil-immersion, the examination of the intestine with the contained flagellates can be carried out in considerable detail, and the following are some of the observations which have been made.

The degree of infection and abundance of the flagellates varies considerably from worm to worm, but in any given specimen the organisms appear to be mainly of the same size and shape. In the case of three or four worms, however, both long and short flagellates were found together. In some cases there are only a few flagellates, whilst in others the lumen of the gut seems to be literally packed with them, so that they appear

as a seething mass. They can be most easily studied in moderately heavily infected worms, where it is generally found that the flagellates assemble in greater number towards the anterior end of the intestine. Here the individual organisms can be focussed more clearly.

A little posterior to the junction of the œsophagus with the intestine the latter often swells out laterally and the lumen is also found to be fairly wide and capacious, and in this region the flagellates can be seen rapidly swimming to and fro, twisting and turning and actively jostling one another. When the nematode bends a little to either side the intestine is thereby flexed and compressed, with the result that its liquid contents are caused to flow rapidly backwards and forwards. The flagellates are carried passively by the stream without being able to resist or swim against the current. In the case of worms with a light infection of from one or two to six flagellates, the latter seem to swim up and down the intestine freely without assembling so definitely towards the anterior end.

In watching the flagellates moving within the intestine an interesting feature in the anatomy of the worms is revealed. This is the lining membrane of the intestine, which appears to be composed of a smooth hyaline layer, flexible but impenetrable to solid particles. The flagellates swim up against and along it, but they never seem to become attached to it, nor do they ever seem to get through it into the granular contents of the intestinal cells.

The shape, dimensions and structure of the flagellates are dealt with in detail later on in this paper, but it may be mentioned here that they are most commonly fusiform in shape, about 10μ or 12μ in length by 1μ to $2 \cdot 5\mu$ in breadth and the tail often tapers finely. The single flagellum can quite easily be seen when a quiet organism is focused, and appears to be about as long as the body. The protoplasm of the body is practically homogeneous in appearance, and occasionally a few small scattered granules, whose nature has not yet been determined, can be seen within it.

Bütschli described and figured rosette formation of the flagellates found by him in the gut of *Trilobus gracilis*, but we have never seen a similar phenomenon in any of the large number of infected *Diplogaster longicauda* examined by us.

Another point of considerable interest is that in most of the infected

adult nematodes the intestine contains bacteria in addition to the flagellates. By carefully focusing under the oil-immersion large numbers of bacteria, mostly longish rods and somewhat shorter forms, can be seen adherent to the hyaline lining membrane of the lumen. When the flagellates swim to and fro or are rapidly carried along in the gut contents, as already described, the bacteria remain in position as though firmly fixed to the membrane. Very often a large tuft of bacteria may be found attached to the wall of the intestine just at the junction of the latter with the œsophagus, and large numbers of them can frequently be seen packing the rather dilated lumen of the second œsophageal bulb. These apparently living bacteria are also not detached by the movements of the flagellates, nor by the pumping motion of the œsophagus, which has often been seen in action.

As far as one can form an opinion on such a point from observations on the living worms, they do not appear to be adversely affected by the presence of small or large numbers of flagellates in the gut. The reserve food granules and oil globules in the intestinal cells are plentiful, and the gonad in both sexes has every appearance of being in a healthy condition.

Little is known concerning the real nature of the food of such saprophytic nematodes as *Diplogaster longicauda*, and very little information is available as to the physiology of the digestive processes and the metabolism of the reserve food substances; but the present observations may help indirectly to throw some light on these obscure phenomena. It would seem as though the nematodes are not pouring out active digestive ferments capable of destroying the flagellates and bacteria present in the intestine. Even if they are present it is evident from the abundance of the flagellates, and from the fact that we have seen dividing forms in the living worms, that they are admirably adapted to their surroundings, and that they find the intestinal contents a congenial medium for growth and multiplication. We may therefore probably regard them as non-pathogenic parasites.

Observations on Contractile Vacuoles.

Bütschli (*loc. cit.*) figured and described a contractile vacuole in the flagellate from *Trilobus gracilis* occurring towards the anterior end of the body, but he does not say whether he observed the vacuole in flagellates

whilst within the intestine of the worm, or only when outside the body. The significance of the last remark will appear presently.

During prolonged observations on the living flagellates within the gut of *Diplogaster longicauda* we have never really convinced ourselves of the presence of a contractile vacuole. We are not prepared to claim that it does not occur in flagellates within the gut, but, if present, it has so far escaped our observation. We are paying close attention to this matter in our further work and may have something to say on the matter at a later time. In the case of flagellates outside the body a contractile vacuole has been seen pulsating, as the following account shows.

Occasionally the organisms have been seen in the process of being shot out from the anus, possibly under the pressure of the cover-slip; and it has proved of interest to watch the behaviour of these released forms. It seems clear that the liquid surrounding the worms, which has generally been some of the decayed bulb material plus a little tap-water, is not so favourable a medium for the continued life of the flagellates as the contents of the intestine. Sometimes a flagellate on reaching the exterior would lash about for a short time, and then the body would begin to round up and finally become quite inactive. Bütschli says that the flagellates from *Trilobus gracilis* behaved in practically the same way and died rather quickly in water.

In other preparations the flagellates after being expelled from the worm retained their characteristic shape for some considerable time, up to thirty minutes, and could be observed swimming fairly slowly. On carefully watching one or two of these a contractile vacuole was seen to make its appearance towards the anterior end of the body and then to collapse suddenly. The process of diastole and systole was timed, and in one organism the vacuole was seen to appear and disappear four times in six minutes. We have both observed these vacuoles on different occasions and have confirmed each other's observations.

The point is of interest, for amongst the parasitic flagellates and amœbæ, and amongst the marine amœbæ, contractile vacuoles are said not to be present. Our observations also link up with those of Hogue (1923), who found that in an amœba, *Vahlkampfia calkensi*, from the marine oyster contractile vacuoles were not present, but when the organism was cultured on a fresh-water medium it developed one or more contractile vacuoles. Her view is that with the change of environ-

ment the contractile vacuole is developed so as to provide an organ whereby the amœba attempts to regulate the osmotic pressure of the protoplasm in relation to the new surroundings. Such a view would seem to fit our observations also, for the contractile vacuole only appears when the flagellate is outside the worm and is apparently trying to accommodate itself to a new and unfavourable environment.

Morphology of the Flagellates.

The morphology of the flagellates was studied both from living specimens and from stained preparations. The latter were obtained by crushing infected worms between two cover-slips, thus forming a smear of the intestinal contents. Difficulty was experienced in getting the flagellates to stick to the cover-slips, but a little fresh blood-serum, placed on the slip before the nematodes were crushed, ensured their adherence. Both wet and dry fixation methods were employed. Heidenhain's iron hæmatoxylin following fixation in Schaudinn's fluid, and Giemsa stain following fixation with osmic acid vapour and methyl alcohol, were found to give the best results, and the following morphological descriptions have been compiled chiefly from films prepared by these two methods.

Before describing in detail the various types of flagellate present in the host at different periods of infection, it seems advisable to give some account of those morphological characters which are common to them all. The cytoplasm, which is of fairly deeply staining character, contains granules with a marked affinity for chromatin dyes. These granules, which are of two types, consist of small dots irregularly scattered through the cytoplasm, and larger bodies, fewer in number, which are usually confined to the posterior region of the body. It seems probable that these granules are composed of volutin or some similar substance, and serve the purpose of reserve food bodies. The nucleus is of the vesicular type. The karysome varies in size and shape ; though most commonly spherical and centrally placed, it is occasionally rod-shaped, crescentic, or composed of two or three rounded masses either closely applied, or connected together by thread-like structures. These latter forms, which are chiefly found in flagellates undergoing division, probably represent early division stages of the nucleus. The kinetoplast is of the usual type with a large, rod-shaped or oval parabasal body and an anterior centrosome from which the axoneme takes its origin. The free

portion of the flagellum is of considerable thickness and shows comparatively slight variation in length, ranging usually only from 8μ to 12μ.

To determine the incidence of the various flagellate types at different periods during the course of the infection, the following method was

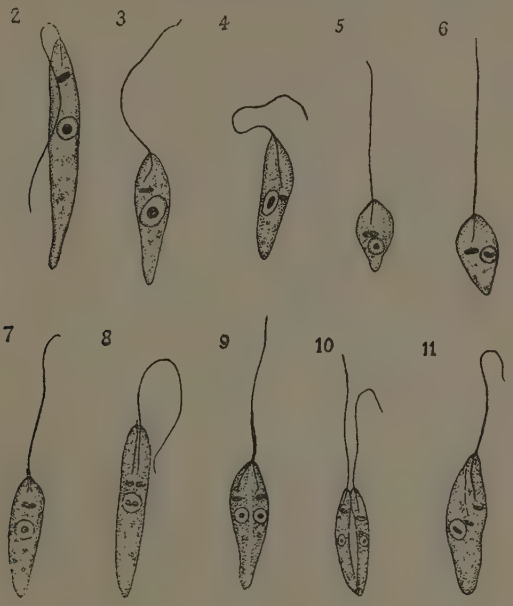

Fig. 2.—Elongated leptomonad form. (×2,500.)
Fig. 3.—Flagellate of intermediate type. (×2,500.)
Fig. 4.—Crithidial type of flagellate, large form. (×2,500.)
Figs. 5 and 6.—Crithidial type of flagellate, small forms. (×2,500.)
Figs. 7 to 10.—Stages of division of leptomonad form. (×2,500.)
Fig. 11.—Early division stage of crithidial form. (×2,500.)

adopted. Infected worms were isolated, examined, and graded into three groups, namely slight, medium and heavy infections. Those placed in the " slight infection " group contained from one to six flagellates only. In the majority of these only two or three active flagellates could be distinguished moving about within the gut. " Medium infections "

included worms which were seen to contain approximately twenty active forms. At this stage the flagellates are not confined to the fore-part of the intestine, but move of their own accord from the anterior to the posterior end. In the third group are included two types of infestation, worms in which the flagellates are very numerous but are confined to the anterior end of the intestine, and worms in which a heavy infection extends from the anterior end as far back as the anus.

Films made from these three groups were studied separately, and careful morphological comparisons of the contained flagellates were made. The size and shape of the body and the relative positions of the nuclear structures formed the chief differentiating factors, and on these the types described below are based.

No morphological distinctions are found between the flagellates present in " slight " and " medium " infections of the host. In both these groups two distinct flagellate types can be distinguished, but the majority of the individual organisms are intermediate in structure between these two extremes. Slender forms of the true leptomonad type with the kinetoplast situated a considerable distance anterior to the nucleus are fairly numerous. These measure from $6 \cdot 8\mu$ to 11μ in length by 1μ to $1 \cdot 2\mu$ in width. Stouter forms, approaching the crithidial type of structure, form a smaller proportion of the infecting organisms. In these the kinetoplast is displaced posteriorly and lies either closely adjacent to the anterior region of the nuclear membrane or else even further back and in the same lateral plane as the nucleus. The flagellum in these forms is discernible passing back through the body as the axoneme from the anterior end to its origin at the kinetoplast, but does not appear to lie on the surface of the cytoplasm, and there is no trace of an undulating membrane. These forms vary from 8μ to $10 \cdot 4\mu$ in length by 2μ to $2 \cdot 4\mu$ in breadth. As already stated, forms of intermediate structure are more numerous than either of the foregoing types. The parabasal body is situated some distance from the anterior extremity of the body, but is not closely approximated to the nucleus. It is, however, usually arranged somewhat laterally in the body. These forms are also intermediate in size, ranging between 7μ and 10μ in length and $1 \cdot 2\mu$ and $1 \cdot 6\mu$ in width.

In the third group of the series, in which the infections are heavier

and presumably of longer duration, greater morphological variations are present, both between the individuals infecting a single host, and between infections considered as a whole. True leptomonad types ranged from 8μ to 14μ in length and 1μ to $2\cdot4\mu$ in width. Crithidial types and intermediate types similar to those described above are the most numerous, and, in addition to these, very small forms may also be present. These small forms measure from $4\cdot8\mu$ to $5\cdot2\mu$ in length and from 2μ to $2\cdot4\mu$ in breadth. They are either ovoid or, more usually, pear-shaped, the posterior half of the body being narrow and ending in a blunt point, the anterior half broad, but drawn out into a fine point in the region of the insertion of the flagellum. The nucleus in these forms is situated somewhat laterally in the body and midway between the anterior and posterior ends. The large rod-shaped parabasal body lies either parallel to the nucleus or just in front of it, and is closely applied to the nuclear membrane. Again no trace of an undulating membrane can be distinguished, nor does the axoneme appear to be situated on the surface of the body. Several of the films made from heavily infected hosts contained a few of these short, stumpy forms, which were also observed in the living state in preparations made in water and saline, but in two cases of long standing infection these were found to be the predominating form. Although these forms suggest a transition stage between flagellate and non-flagellate leishmania stages, careful searching has so far failed to reveal a true leishmania stage, and in no case was any reduction in the length of the flagellum observed.

Dividing forms were seen amongst both leptomonad and crithidial types, but were not very numerous. As slightly infected worms kept under observation were found after a period of twelve hours to become very heavily infested, this scarcity of division stages in the films points to the conclusion that the process of division is rapid in its completion. Division of the body is, as usual, preceded by division of the kinetoplast, accompanied by the outgrowth of a new flagellum and the division of the nucleus. A point worthy of note with regard to dividing forms is the comparative frequency of forms such as that shown in fig. 9, where the nuclear elements and flagellum are reduplicated but the body remains entire, showing, however, a thin non-granular faintly staining strip extending down the middle of the body, the presumable ultimate line of fission.

Remarks on Systematic Position.

The flagellates found by Bütschli were not named by him, but were subsequently named by Saville Kent (1880) *Leptomonas bütschlii* as type of the genus.

As Wenyon (1926, p. 348) points out, this organism has never been studied in the light of present knowledge, and we are ignorant on the details of its internal structure. The flagellates in *Diplogaster longicauda* studied by us agree with *L. bütschlii* in general shape and size, but not in the length of the flagellum, but beyond this, of course, we cannot go at present. It is quite possible that both the flagellates are the same species, but until *Leptomonas bütschlii* has been found in *Trilobus gracilis* and studied afresh by modern cytological methods the identity of the two organisms cannot be definitely determined and remains conjectural.

Wenyon (*loc. cit.*) defines the genus *Leptomonas* as including flagellates which in their life-cycle exhibit both leishmania and leptomonas forms, and which are confined to invertebrate hosts. Our organisms certainly exhibit the leptomonas stage, but so far we have not succeeded in finding true leishmania forms. We are, however, continuing our investigations of these interesting flagellates both in the direction of transmission experiments to other nematodes and in cultures on artificial media, and when we have more information on these and other points we may be in a better position to express an opinion on the question of the systematic position of the organism and its proper name. At the present time we prefer to leave the question of the name an open one.

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Observations on the Oöcysts of Coccidia found in the Fæces of Carnivores.

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Introduction.

From a study of the literature dealing with the coccidial parasites of the *Carnivora*, it appears that, while much work has been done on the coccidia of the domesticated dog and cat, comparatively few authors have reported the occurrence of these parasites in the fæces of undomesticated representatives of the Order. Examinations of material recently made available from various sources have revealed the presence of coccidial infections in several hosts from which these parasites have not previously been reported. The purpose of the present paper is, therefore, to record these observations and to compare them with the findings of other authors.

For access to the material of the first case dealt with below I am indebted to Mr. J. R. Cunningham, B.V.Sc., M.R.C.V.S., who discovered in the fæces of foxes bred for fur on Prince Edward Island, Canada, the oöcysts of a coccidium, which he sent to this Institute for investigation and specific diagnosis.

Isospora from the Fæces of a Fox.

The oöcysts in this case were very numerous and were uniform in type. They were ovoid, and measured from $12 \cdot 5\mu$ to 14μ in length, and from $11 \cdot 5\mu$ to 13μ in breadth. The average size was $13 \cdot 5\mu$ by $12 \cdot 5\mu$.

The cyst wall, which was clear and colourless, consisted of the usual three layers. The middle layer measured $0 \cdot 5\mu$ in thickness, and the inner and outer membranes were so fine as to be almost indistinguishable. In no case could a micropyle be detected.

The protoplasmic contents showed all stages of development from a

single spherical mass to fully developed sporocysts containing sporozoites. In the undeveloped form the granular cytoplasmic mass measured about 10μ in diameter and was centrally placed within the oöcyst. The nucleus could not be distinguished. The next stage showed two oval sporoblasts which were developed without an oöcystic residuum. In these, again, the granular cytoplasm completely masked the nuclei.

The sporocysts were ovoid, with double contoured walls, and measured about 9μ in length by 7μ in breadth. When fully mature they contained four club-shaped sporozoites and a large rounded mass of residual cytoplasm. The sporozoites were slightly curved and were arranged obliquely to the long axis of the sporocyst, with their broader ends together near one pole. The cytoplasmic residuum constantly occupied the opposite pole of the sporocyst, thus determining the oblique arrangement of the sporozoites.

The oöcysts were kept at room temperature in normal saline solution and in 2·5 per cent. formalin in which they had been originally packed. Development of the immature forms was found to continue over a period of ten days, by which time the majority had reached maturity. A few, which remained with the contents rounded up in a single mass, were probably unfertilised. Some of the oöcysts in formalin were arrested at various stages of development. This was probably due to injuries to the cyst wall having occurred during transit from Canada. An attempt was made to infect a cat by feeding it on material heavily infected with fully developed oöcysts, but this remained unsuccessful.

Morphologically this parasite closely resembles *Isospora bigemina* Stiles 1891, to which species it most probably belongs. There are, however, several minor points of difference which should be noted. As may be seen by reference to the table of measurements given by Wenyon (1923) the oöcysts described above, while conforming in length with cases of *I. bigemina* from cats and dogs, tend to be somewhat broader. Further, Wenyon described the walls of the sporocysts of *I. bigemina* as being fairly thick and double contoured, while the oöcyst wall was of a more delicate nature. In the oöcyst from the fox this condition was reversed, for although the sporocyst walls were double contoured they were much thinner than the wall of the oöcysts, of which, the middle layer, as stated above, measured 0·5μ in thickness. Unfortunately it is not known whether, when freshly passed, the oöcysts

were at a uniform stage of development. This, however, seems unlikely, since, on arrival in England some were fully developed, containing sporozoites, while others attained maturity only after being kept for ten days.

Isospora from the Fæces of a Lynx.

On examination of the rectal contents of a lynx which died in the Zoological Gardens of London, a very heavy infection of coccidial oöcysts was discovered. These were oval in shape and varied from 40μ to 47μ in length and from 28μ to 36·5μ in breadth. The middle layer of the oöcyst wall measured about 0·75μ in thickness and was of a slightly yellowish tint. The micropyle showed as a distinct gap in this layer of 4μ to 5μ in diameter.

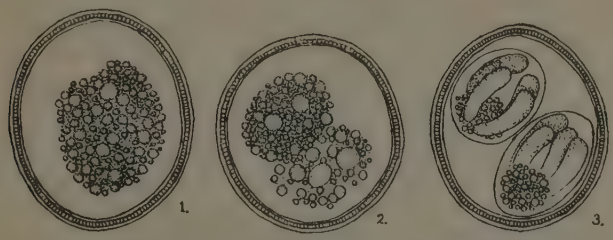

Fig. 1.—Unsegmented oöcyst from the fæces of a fox. Fig. 2.—Oöcyst at sporoblastic stage. Fig. 3.—Fully developed oöcyst. (× 2,000.)

In every example the protoplasmic contents were rounded up to form a central spherical mass. This consisted of highly granular cytoplasm, in which, however, the nucleus could be distinguished as a clear rounded area of about 5μ diameter. Development took place when the cysts were kept at room temperature, and was completed in from seven to nine days. Two oval sporoblasts were formed without a residuum. The sporocysts were regularly ovoid with double contoured walls, and measured from 20μ to 23μ in length by 14μ to 17·5μ in breadth. When fully developed each contained four elongated sporozoites and a considerable mass of residual cytoplasm. The sporozoites were of the usual type, with one end bluntly rounded, the other slightly narrower. They were about 15μ in length. The broad end of each was occupied by a large greenish globular body, while a similar but smaller body

was also sometimes present in the narrower extremity. The nuclei were centrally placed and of the spherical vesicular type.

The oöcysts of this coccidium are morphologically identical with those of *Isospora felis* Wenyon 1923. No material was available for a study of other stages of the life-cycle and no transmission experiments were carried out.

Isospora from the Fæces of an Eyot-cat.

This material was also obtained from the Zoological Gardens, and again no tissues were available for sectioning, so that the morphology of the major portion of the life-cycle remains unknown.

The infection was apparently a heavy one, the oöcysts being abundant in the fæces. They were ovoid in form and showed a slight variation in size, ranging from 20μ to 26μ in the long axis by 14μ to 18μ in the short axis. The wall of the oöcyst was colourless, the middle layer measuring 0·5μ in thickness. The micropyle was very distinct and about 3μ in width.

When freshly passed the oöcysts were undeveloped, with the contents rounded up into a more or less centrally placed spherical mass. With the formation of the sporoblasts a small but constant oöcystic residuum was formed. The sporocysts were ovoid, with double contoured walls and varied from 11μ to 13μ in length by 6μ to 8μ in breadth. When fully mature they contained four sporozoites which lay curved around a large, more or less spherical residual mass which usually occupied a central position. The thicker end of each sporozoite contained a greenish refractile globule. The nuclei were difficult to distinguish owing to the large amount of residual material present. They were, however, centrally placed and of the usual type.

In view of the similarity between these oöcysts and the published descriptions of *I. rivolta* Grassi 1879, from the domestic cat and dog, a comparative study was made of these forms. Two cats, naturally infected with *I. rivolta*, were obtained and the oöcysts found in their fæces were compared at all stages of development with those from the eyot-cat. Only two points of difference were thus discovered. Firstly, the range in size of the oöcysts was slightly greater in the domestic cats than in the eyot-cat. Secondly, while a small oöcystic residuum was constantly present in the form from the eyot-cat, it varied in size and was sometimes entirely lacking in the oöcysts from the domestic cats. It

seems, however, highly probable that the case described above is one of *I. rivolta* occurring in a new host.

Other Coccidial Infections in Carnivores.

Only four other references to coccidial parasites occurring in undomesticated carnivores can be traced in the literature. Railliet and Lucet (1890) described coccidial oöcysts in the intestinal tissues of the pole-cat (*Mustela putorius*). This parasite differed only slightly in size from the form in the dog to which Stiles had given the name of *Coccidium bigeminum*. Hence, in a later paper, Railliet and Lucet named the form in the pole-cat *Coccidium bigeminum* var. *putori* as distinct from *C. bigeminum* var. *canis* and *C. bigeminum* var. *cati* in the dog and cat respectively. These varieties were based on the size of the sporocysts, which were as follows :—

C. bigeminum var. *canis*—sporocysts 12μ to 15μ by 7μ to 9μ.

C. bigeminum var. *cati*—sporocysts 8μ to 10μ by 7μ to 9μ.

C. bigeminum var. *putori*—sporocysts 8μ to 12μ by 6μ to 8μ.

It has since been pointed out by Wasielewski (1904) and Wenyon (1923) that these dimensional variations are insufficient to vindicate the formation of varieties. Thus, according to the rules of nomenclature, the parasite of the pole-cat should be known as *Isospora bigemina* Stiles 1891.

Weidmann (1915) described coccidial oöcysts from the fæces of swift-foxes in the Western United States. These were elliptical, varying from 25μ to 40μ in length by 25μ to 30μ in breadth. The sporocysts varied in length from 14μ to 16μ, but their width was not stated. Weidmann considered these oöcysts to form a variety of *C. bigeminum* and advanced the varietal name "*canivecolis*." In his paper "The Coccidiosis of Cats and Dogs and the Status of the *Isospora* of Man," Wenyon summed up the position with regard to this case in the following words :—"This parasite, which is certainly not a variety of *Isospora bigemina*, may be identical with *Isospora felis*, but on the other hand it may be distinct. In the latter case the name *Isospora canivecolis* would be correct."

Möller (1923) reported oöcysts in the fæces of two lion cubs kept in captivity in the Zoological Gardens of Berlin. These oöcysts, which measured 36μ to 48μ by 28μ to 34μ, average size 42μ by 30μ, belonged to the genus *Isospora*, but their development was not followed beyond

the sporoblastic stage. The author drew attention to their morphological similarity to *I. felis* Wenyon 1923.

Finally, Adler (1924) described an *Isospora* pathogenic in civet cats. This parasite was confined to the lower half of the small intestine. The developmental stages were fully described and do not differ in any essential feature from those of *I. rivolta* as described by Zapfe. In size and general morphology the oöcysts also approximated to *I. rivolta*, but they differed from the latter in being passed containing two sporoblasts instead of in the unsegmented condition. Experimental transmission of this parasite to cats, kittens and young dogs was attempted by means of administering heavily infected food. These experiments, however, all proved unsuccessful. Adler therefore concluded that the parasite of civet cats was not identical with *Isospora rivolta*, and proposed for it the name *Isospora viverræ*.

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Some Cephaline Gregarines parasitic in British Myriopods.

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The myriopod hosts of the Gregarines described below were collected by a member of this Institute and identified by the Rev. S. Graham Brade-Birks, to both of whom I wish here to express acknowledgment and thanks. All the material was received in a preserved condition and the majority consisted of gut-contents only, so that no material was available for sectioning with a view to the discovery of intracellular stages. A certain amount of shrinkage had taken place in some instances, and it seems probable that the colour factor has been, to a large extent, lost. All the following descriptions have, however, been compiled from undistorted specimens selected after a careful comparative study of many examples. The ratios of dimensions have been made in every case from a single large typical sporont, and have been controlled by average ratios of from six to ten other typical examples. Unfortunately representative stages of the three phases of the life-cycle were in no instance discovered, and the following descriptions are, therefore, necessarily incomplete. The parasites have, however, been classified and identified as far as possible from the available data.

(1) Host—*Glomeris marginata* (Villers).

The sporonts were solitary, ranging in length from 300μ to 500μ. The protomerite was subglobular, divided into two parts, the anterior from the posterior two-thirds, by a slight constriction. These two portions differed in internal structure, the anterior being greenish and free from granules, while the posterior portion was coarsely granular and brownish in colour. A deep constriction was present in the region of the septum.

The deutomerite was elongated, cylindrical, and terminated very bluntly. The epicyte was distinct and the endocyte brown and granular. The nucleus was ellipsoidal, arranged longitudinally in the deutomerite.

Ratios of Dimensions.

Length protomerite	: total length	:: 1 : 17·5
Width protomerite	: width deutomerite	:: 1 : 2·4
Width protomerite	: length protomerite	:: 1 : 0·7
Length protomerite	: length deutomerite	:: 1 : 2·4

Two trophozoites were found lying free in the gut-contents. These were smaller than the sporonts but similar in general form. The protomerite of the trophozoite was not constricted, but the anterior end was concave, forming a shallow pit within which the epimerite was inserted. The epimerite was a simple, sessile, globular knob projecting from the anterior concavity of the protomerite. In the trophozoites the granular portion of the protomerite occupied only the posterior third, the anterior two-thirds being clear around the periphery, but containing a very finely granular cone-shaped core extending from the posterior region to the point of insertion of the epimerite. No trophozoites were found either attached to or within the epithelial cells of the gut. Cysts and spores were not seen.

This Gregarine shows a close morphological resemblance to *Cnemidospora lutea* Schneider 1882 from the intestine of *Glomeris* sp. taken at Poitiers, France. It differs from Schneider's parasite only slightly in dimensions. The maximum length, given by Schneider, is 500μ, while the ratios of the various parts are as follows:

Length protomerite	: total length	:: 1 : 15
Width protomerite	: width deutomerite	:: 1 : 1·6
Width protomerite	: Length protomerite	:: 4 : 3

Neither trophozoites nor cysts were described, but the spores were noted as being ellipsoidal with a thick integument.

It seems safe to assume that the present case represents an infection of *Cnemidospora lutea* Schneider 1882, and the morphological characters of the trophozoite are therefore added to the data supplied in the original description of this species.

(2) Host—*Polydesmus* " *complanatus* " of English Authors (*ne* Linné)

The sporonts were solitary, ovidal or elongated, varying in length

from 100μ to 184μ and in breadth from 30μ to 70μ. The protomerite was short, forming a ring superimposed on the deutomerite, through the centre of which the septum projected. The epicyte of the protomerite was distinct and the endocyte sparsely granular. There was a slight constriction at the septum. The deutomerite widened gradually from the septum throughout its anterior third, then gradually tapered to a blunt termination. The endocyte was greyish, containing a plainly visible, spherical nucleus with a single large karyosome, centrally situated in the widest region of the body.

Ratios of Dimensions.

Length protomerite	: total length	::	1 : 29·3
Width protomerite	: width deutomerite	::	1 : 2
Width protomerite	: length protomerite	::	1 : 0·22
Length protomerite	: length deutomerite	::	1 : 30

The epimerite, which consisted of a simple globular or elongated knob, was attached to the apex of the projecting septum and not to the protomerite. Neither cysts nor spores were seen.

This Gregarine, from the characters of the protomerite and epimerite, obviously belongs to the genus *Amphoroides* ; it differs, however, in several respects from the two species of this genus hitherto described. Of these, *A. polydesmi* (Léger 1892) Labbé 1899 shows the protomerite in the form of a deeply crenulate collar surrounding the septum. The deutomerite is cylindrical in its anterior third ; it then widens appreciably to form a shoulder, behind which it tapers gradually to end in a broad flattened extremity of approximately the same width as the anterior third. The ratios of the dimensions also show great discrepancy from those cited above.

A. calverti (Crawley 1903) Watson 1916 is a much larger form, the average size of the sporonts being 1,400μ by 120μ. The deutomerite of this species is elongated, tapering to a sharp point.

The form described in the present paper appears, therefore, to belong to a species hitherto undescribed, for which the name *Amphoroides circi* is suggested.

(3) Host—*Cylindroiulus punctatus* (Leach).

Numerous sporonts and a single trophozoite of a Gregarine were

discovered in the preserved gut-contents of this myriopod. As no complete specimens of the host were obtainable the younger stages of the development were not observed.

The sporonts were solitary, small forms ovoid, larger forms sub-globular in shape. In length the sporonts varied from 40μ to 100μ and in width from 26μ to 85μ. In the younger forms the protomerite was cylindrical at the base and conical above. In the mature sporonts the protomerite, though only slightly larger than in the young forms, was almost hemispherical in shape with a very slightly pointed anterior extremity. A slight constriction at the septum was visible in the young forms only. The deutomerite was broadly ovoid to sub-globular. The epicyte was distinct, endocyte dark grey throughout and uniformly granular. The nucleus, which was obscured or only faintly visible in unstained specimens, was spherical or ovoid, with a single large karyosome, and varied greatly in position within the deutomerite.

Ratios of Dimensions.

Length protomerite	: total length	::	1 : 6
Width protomerite	: width deutomerite	::	1 : 3
Width protomerite	: length protomerite	::	1 : 0·4
Length protomerite	: length deutomerite	::	1 : 8·4

The single trophozoite which was discovered was similar in general form and structure to the young sporonts. The epimerite was a simple cylindrical structure. Neither cysts not spores were seen.

The systematic position of this Gregarine remains obscure. None of the named genera of cephaline Gregarines are characterised by the possession of a simple cylindrical epimerite and solitary sporonts. In 1900 Laveran and Mesnil described a Gregarine, *Pyxinia frenzeli*, from the intestine of *Attegenus pellio* and *Dermestes* sp., which shows a strong morphological similarity to the species described above. The epimerite of *P. frenzeli* is described and figured as consisting of a slender cylindrical base, equal in length to the protomerite, and bearing a short sharp apical style. This suggests the possibility that the parasite of *Cylindroiulus punctatus* belongs to this genus, and probably represents the same species, and that, in the case of the single trophozoite which was discovered, the epimerite had been mutilated in its removal from the epithelium. Unfortunately Laveran and Mesnil figured only the

Figs. 1–2.—*Cnemidospora lutea*. Fig. 1, Protomerite of sporont. Fig. 2, Protomerite of trophozoite showing epimerite.

Figs. 3–5.—*Amphoroides circi* n. sp. Fig. 3, Sporont. Fig. 4, Anterior end of sporont. Fig. 5, End of trophozoite, showing epimerite attached to cell of host.

Fig. 6.—Mature sporont from *Cylindroiulus punctatus*.

Figs. 7–9.—*Actinocephalus blanuli* n. sp. Fig. 7, Mature sporont. Fig. 8, Protomerite of sporont. Fig. 9, End of trophozoite, showing epimerite.

Figs. 10–11.—Type A parasite from *Tachypodoiulus niger*. Fig. 10, Mature sporont. Fig. 11, Anterior end of sporont.

Fig. 12.—Gregarine type B from *Tachypodoiulus niger*—sporont.

Figs. 13–14.—Gregarine type C from *Tachypodoiulus niger*. Fig. 13, Sporont. Fig. 14, End of trophozoite showing epimerite attached to cell of host.

trophozoite of *P. frenzeli*. Cysts were not seen. The spores were ovoidal, measuring 14μ by 6μ.

(4) Host—*Blaniulus guttulatus* (Bosc.).

A Gregarine was found in the gut of this myriopod which showed a distinct dimorphism of the sporonts. They varied from slender and elongated forms to short, stouter ovoid forms. The elongated forms were most numerous and varied in length from 35μ to 200μ and in breadth from 17μ to 52μ. The ovoid forms were intermediate in size. Numerous examples showing various gradations in structure between the extreme types were found, and led to the conclusion that only a single species was represented.

In the long forms the protomerite was regularly or irregularly hemispherical. There was no constriction at the septum. The deutomerite widened rapidly through about the anterior third of its length, then gradually narrowed to end bluntly, the posterior extremity being from three-quarters to one and a half times as wide as the septal region.

Ratios of Dimensions.

Length protomerite	: total length	::	1 : 17
Width protomerite	: width deutomerite	::	1 : 2·5
Width protomerite	: length protomerite	::	1 : 0·5
Length protomerite	: length deutomerite	::	1 : 4

In the ovoid forms the protomerite varied from conoidal to hemispherical in shape, and there was a slight constriction at the septum. The deutomerite was ovoid, slightly broader at the anterior than at the posterior end.

Ratios of Dimensions.

Length protomerite	: total length	::	1 : 4·5
Width protomerite	: width deutomerite	::	1 : 2
Width protomerite	: length protomerite	::	1 : 0·7
Length protomerite	: length deutomerite	::	1 : 3·5

From a comparison of these two tables of ratios it will be seen that the only marked discrepancy lies in the ratio between the length of the protomerite and the total length of the parasite. The cytological structure of the two forms was identical. The epicyte was distinct,

and the endocyte granular, opaque, and in reflected light white in colour. The nucleus was faintly visible, spherical or ovoid, with a single large karyosome. In the bulk of the protomerite the endocytic granules were sparse but large and distinct ; at the extreme anterior end, however, they were very numerous and of extreme delicacy.

The trophozoites showed a dimorphism similar to that described as occurring in the sporonts, with which, except for a smaller range in size and the presence of the epimerite, they were morphologically identical. The epimerite consisted of a short neck-like base expanding into a very delicate cup-shaped structure divided marginally into four lobes. Cysts and spores were not seen.

From the morphological characters of the trophozoite and sporont it appears that this parasite should be placed in the genus *Actinocephalus* Stein 1848. It does not, however, conform to any of the species hitherto described. The writer proposes, therefore, to create a new species, *Actinocephalus blanuli*, of which the parasite described above is the type.

(5) Host—*Tachypodoiulus niger* (Leach).

Three forms were present in this host, all morphologically distinct and probably representing different genera.

Type A.

The sporonts were solitary, elongated, and slender. Their maximum length was 230μ, maximum breadth 34μ. The protomerite was rounded with a broad nipple-like projection at the anterior end. The epicyte of the protomerite was distinct and consisted of two layers. The outer layer, which was of uniform thickness, surrounded the whole protomerite. Within this was a second layer, of apparently identical structure and consistency, which was slightly thickened over the septum and again, more markedly so, at the anterior end, where, however, it was discontinuous, forming a thickened ring-like structure with a central cavity, supporting the walls of the nipple-like projection. A further peculiarity of the epicyte in this form consisted of a series of three ring-like markings which encircled the anterior projection of the protomerite and apparently extended through the substance of both layers of epicyte, the internal layer in many cases being slightly indented where these lines intersected it.

In the deutomerite the epicyte was distinct and single. The endocyte was uniformly granular in both protomerite and deutomerite. There was a slight constriction at the septum. The deutomerite was cylindrical or very slightly tapering through the anterior four-fifths of its length. It then narrowed abruptly to about four-sevenths of its maximum width, again became cylindrical, and terminated very bluntly. The nucleus was ovoid, arranged longitudinally, but in no definite position within the deutomerite, and contained a single large ovoid karyosome eccentrically placed.

Ratios of Dimensions.

Length protomerite	:	total length	:	1 : 7·8
Width protomerite	:	width deutomerite	::	1 : 1·2
Width protomerite	:	length protomerite	::	1 : 1·02
Length protomerite	:	length deutomerite	::	1 : 6·4

Trophozoites, cysts and spores were not seen.

Type B.

These sporonts were solitary and ovoid, with a maximum length of 120μ and maximum width of 60μ. The protomerite was cone-shaped, with epicyte distinct and endocyte uniformly granular. A slight constriction was present at the septum. The deutomerite was ovoid, tapering slightly at the distal extremity. The endocyte was granular with the granules arranged in the form of a coarse network. The nucleus was plainly visible, spherical or ovoid, and contained a single large karyosome. No trophozoites were seen.

Ratios of Dimensions.

Length protomerite	:	total length	::	1 : 6·5
Width protomerite	:	width deutomerite	::	1 : 1·9
Width protomerite	:	length protomerite	::	1 : 1·4
Length protomerite	:	length deutomerite	::	1 : 5·5

Sporonts of this type were found in various early stages of encystment, but as all the material was preserved no mature cystic stages were obtained. The cysts were spherical, of about 125μ diameter. The cyst-wall was translucent and colourless, consisting of a dense inner layer surrounded

by numerous concentric rings, of a transparent material, to the outermost of which a considerable amount of debris was usually adherent. The two conjugants could in every case be clearly distinguished, and no stages of gamete formation were discovered.

Type C.

Sporonts of this type were less numerous than either of the two foregoing types, but about twenty were discovered and studied. They were solitary, and ovoid to spherical in shape. Their maximum length was 64μ and maximum width 53μ. The protomerite was large, globose or almost rectangular, and was sunk from half to two-thirds its length within a deep invagination at the anterior end of the deutomerite. The deutomerite was rounded or ovoid, with a wide and deep anterior depression which contained the protomerite. The epicyte was distinct and the endocyte finely and uniformly granular. The nucleus, which was clearly visible, was large, ovoid, arranged transversely in the deutomerite, and contained a single karyosome.

Ratios of Dimensions.

Length protomerite	:	total length	::	1 : 3·1
Width protomerite	:	width deutomerite	::	1 : 2
Width protomerite	:	length protomerite	::	1 : 0·8
Length protomerite	:	length deutomerite	::	1 : 2·7

The trophozoites were morphologically similar to the sporonts, but did not attain the maximum dimensions of the latter. The epimerite was a short, globose structure, the width of which exceeded the length in the ratio of 5 : 4. It was sunk for about three-quarters of its length within a deep pit in the protomerite. The method of insertion differed from the method of insertion of the protomerite within the deutomerite, in that the lateral walls of the pit were closely applied to the epimerite, whereas, between the protomerite and deutomerite a distinct lateral space was constantly present.

None of the three forms described above can be identified with any of the named genera of cephaline Gregarines, but until further investigations into their morphology and life-cycles have been carried out it seems inadvisable to create new genera.

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Note on the Occurrence of a Sarcocyst, parasitic in a Wallaby.

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During the autopsy of a Bennett's wallaby (*Macropus bennetti*) which died in the Zoological Gardens of London, a number of small white bodies were observed lying embedded in the wall of both large and small intestines. As these proved on examination to contain innumerable spore-like bodies, presumably parasitic in origin, portions of the tissues were sent to this Institute for investigation.

The parasites, which were found to be sarcocysts, were visible as rounded white eminences projecting, some from the mucosal, others from the serous surface of the intestinal wall. The smallest forms, which were roughly spherical, measured about 0·5 mm. in diameter. Larger individuals were ovoidal and varied in size up to 1·75 mm. by 1 mm. as maximum dimensions. On sectioning they were seen to be situated within the muscle layers of the intestine, where they caused considerable distension but no definite lesion, and were accompanied by no apparent tissue reaction on the part of the host. The cysts lying in the longitudinal muscle layer caused projections on the serous surface of the intestine; those lying within the circular layer caused distortion of the mucosa.

The cysts were of the usual type and were all fully developed, the central chambers being empty and partially collapsed while those at the periphery were distended with spores. The spores were sickle-shaped, measuring from 17μ to 19μ in length by 4·5μ to 5μ in width. Morphologically they were indistinguishable from the spores of *Sarcocystis tenella*.

It is interesting to note that, although the parasites in the intestinal wall were plainly visible to the naked eye, microscopic examination of

the other tissues throughout the body showed no indication of any further infection. Unfortunately none of the skeletal muscle was available for sectioning.

An attempt was made to transmit the parasite to a mouse by feeding it with material containing great numbers of the spores. Two months after the infective feed a careful examination was made of the intestinal walls but no parasites were discovered. Sarcocysts were present in the skeletal muscles, but these were probably due to a natural infection of *S. muris.*

No previous record can be found of a sarcocyst infection occurring in a wallaby. A parasite discovered by Blanchard in the submucosa of a kangaroo (*Macropus penicillatus*) was formerly included among the Sarcosporidia as *Sarcocystis mucosa* Blanchard 1885. This, however, as shown by Nöller, belongs to the genus *Globidium* and hence becomes *Globidium mucosa* (Blanchard 1885) Nöller 1920.

Accepting *S. mucosa* as an authentic sarcocyst, Darling, 1910, reported the discovery of the second case of *Sarcocystis* parasitic in a marsupial, the host being an opossum (*Didelphis*). This parasite, *S. darlingi* Brumpt 1913, was present in connective tissue, lungs, glandular tissue, heart, and the smooth muscle-layers of the stomach, œsophagus and small intestine, as well as in the skeletal muscles. In addition to this unusual distribution in the tissues of the host, Darling's parasite differed from the sarcocysts of the higher mammals in being without internal trabeculæ, and hence containing only a single central cavity. These differences, as pointed out by Nöller 1920, suggest the possibility of this parasite belonging to the genus *Globidium* rather than *Sarcocystis*, but further cytological observations are necessary to establish the true systematic position of this form.

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Further Observations on a Flagellate parasitic in the gut of *Diplogaster longicauda*.

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Introduction.

The nematodes containing these flagellates were, as recorded in a previous paper, first discovered in rotting narcissus bulbs. The bulbs, which were heavily attacked by *Tylenchus dipsaci*, had been kept in a moist chamber for several weeks and showed a heavy secondary infection with *Diplogaster longicauda*. After about eight months, when the bulbs had reached an advanced state of decay, the *Diplogaster* infection was found to be dying out. In order to preserve the culture of infected nematodes healthy bulbs, with their growing points removed, were placed in a similar moist chamber and a little of the infected material was added. By repeating this process the culture of infected nematodes has been preserved and a further series of observations has been carried out on the flagellates.

Effects on the Flagellate of the Environment of the Host.

D. longicauda lives and breeds freely in a large number of organic media, in fact in almost any decaying vegetable matter. Sheep's fæces, sterilised and kept well moistened, form a particularly favourable environment.

Cultures of infected *Diplogaster* were set up in various media, including sheep's fæces, moist loam with decaying leaf mould, and decaying gladiolus corm. These cultures were kept under observation and the nematodes were found to multiply freely in every case, but the increase in numbers was most marked where only organic material was present. The effect on the flagellates, however, of these changes of media was found to vary. The culture set up in decaying gladiolus corm retained the infection for a period of four weeks, but during this period the number of infected individuals present gradually diminished, and finally the infection died out. In all other media the flagellates disappeared even sooner, none being discovered after a period of a week to ten days. It is not known whether, in these cases, any transmission of the parasite took place, but in this connection, it was noted that towards the end of the period during which infection persisted the infected nematodes were all in the adult state. This suggests that the infection might possibly be confined to the original worms with which the cultures were set up.

The conclusions led to by these results are that, either the parasite is very susceptible to changes in the surrounding medium, *i.e.*, in the food material taken into the gut of the host, or, that infective forms can only survive outside the body under certain favourable conditions.

In order to discover whether, under such adverse conditions, the flagellates remained in the hosts in a quiescent or resistant state, smears of apparently uninfected worms were made and examined. The results of these examinations were, however, in every case negative. Further, after the infections had apparently died out, subcultures of the nematodes were made in the original medium, but in no case did the infection re-appear.

Effects on the Flagellate of Temperature and Humidity.

About two dozen of the original bulbs in which the infection was found, remained unused, and were kept in a dry but unheated room throughout the winter. Gradual desiccation to a large extent arrested the decay in these. Eight months later half of these bulbs were placed in a moist chamber, and, on examination, were found to contain a large

number of *D. longicauda*, some of which contained the flagellate parasite. The percentage of infection was, however, not so great as that present in the original culture. During a period of two months both the number of *D. longicauda*, and the percentage of infected individuals increased, after which the nematodes gradually died out.

The remainder of the bulbs were left in a dry state throughout the summer and were then placed under moist conditions. In these, although the nematodes were present, the flagellate infection had completely disappeared.

The material from which the second culture was made had been exposed to great variations of temperature, including conditions approaching zero, without either the host or the flagellate being destroyed. The bulbs of the third culture had been subjected to further desiccation but no further extremes of temperature. Desiccation appears, therefore, to be more fatal to the flagellates than low temperature conditions. In killing infected nematodes by heat, it was noticed that, previous to the death of the worm the activity of the flagellates ceased, and they became invisible in the gut, possibly becoming closely applied to the lining membrane.

Experiments were also carried out to determine the effects of excess of moisture on the transmission of the parasite. Cultures of infected nematodes were set up in narcissus bulb medium in varying states of liquidation. The percentage of infection was found to drop in the presence of excess of moisture and to remain more or less stationary, with slight periodic increases, in cultures where only a thin film of moisture was present over the solid medium. Since the multiplication of the nematodes remained approximately the same in every case it was concluded that the infective forms survived more easily outside the body of the host when excess of moisture was not present.

The Morphology of the Parasite.

Flagellates present in the cultures mentioned above were examined, and their morphology was studied, to discover whether any variations were produced as adaptations to varying conditions. In flourishing cultures the flagellates were almost exclusively of the long, slender, leptomonad and crithidial type, among which, in some cases, a few

short stumpy forms were seen. A small proportion of the infected nematodes present in these cultures contained short stumpy forms exclusively. Conversely, in cultures where the infection was dying out, as in the original bulbs after a very advanced stage of decay was reached, fifty per cent. of the infections were found to consist of small forms only, and these were often present in number at the anterior as well as the posterior end of the intestine. Although numerous smears of these individuals were made and examined, no aflagellate stages were found. When liberated from the host and examined in the living condition in tap water, distilled water, extract of narcissus bulb and dilute saline solution, these rounded forms survived for periods varying up to one hour. A contractile vacuole usually appeared within the cytoplasm at the anterior end, but this was not constantly present.

The Course of the Infection within the Host.

A number of worms containing very light infections consisting of one or two flagellates in the fore-gut, were isolated in a favourable medium and subsequently examined daily. The flagellates were found to multiply rapidly during the first forty-eight hours, by which time the anterior portion of the intestine usually contained a seething mass of active parasites. After this period either reproduction was continued until the whole of the gut was invaded, or, more usually, the flagellates gradually migrated to the posterior region of the intestine where stumpy forms appeared amongst them, and, after a further period varying from one to six days, the infection died out. Where multiplication was continued after forty-eight hours it became impossible to distinguish the different types of individual flagellate present, but as the numbers slowly decreased stumpy forms could be distinguished and these remained present until the infection disappeared. Smears of worms from which the parasites were dying or had died out, were examined for resistant stages but these gave negative results.

All the infected nematodes contained some bacteria within the gut. Two varieties of these could commonly be distinguished, one a short form, the other a long rod-shaped organism. In some of the isolated nematodes kept under observation these rod-shaped bacteria were

found to multiply rapidly within twenty-four hours and become motile within the intestine. In these cases the flagellates soon disappeared the bacteria filling up the lumen of the intestine and causing the death of the worm. Stained preparations of these nematodes showed that the bacteria invaded not only the gut but also the body cavity and tissues.

Transmission Experiments.

Various attempts have been made to determine the mode of transmission of the flagellates. Nematodes containing heavy infections of flagellates in the fore-gut were crushed in an extract of rotting bulb material to which clean nematodes were then added. A small piece of bulb was placed in the culture for the worms to feed upon and the medium was kept at the optimum humidity. This experiment was repeated on four occasions, on only one of which did transmission take place, two of the six clean nematodes becoming infected.

Other cultures were made into which several infected nematodes were introduced for forty-eight hours and then removed and replaced by clean individuals. By this method twenty-five per cent. of the clean nematodes became infected. It was noted that, when the infected nematodes were removed after being for forty-eight hours in the medium, several of them were free from flagellates, the latter having presumably passed into the medium.

From these results it seems probable that the motile flagellate stage seen in the intestine is not the usual agent of transmission. This view is supported by previous observations on the length of time these forms survive outside the body. The single successful result of the first series of transmission experiments may have been due to the ingestion of flagellate stages by the nematode, or, on the other hand, aflagellate forms may have been present in this instance, and served to transmit the infection. Such resistant forms, capable of existing for some time outside the body of the host, are, in all probability, developed in the hind region of the intestine from the short rounded forms previously described, whence they pass out into the surrounding medium. They have, however, not yet been demonstrated.

The mites present in the original cultures were dissected and examined,

but neither these not the other species of free-living nematodes which have appeared in the cultures from time to time have been found to contain flagellates.

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Further Observations on the Development of *Globidium gilruthi*.

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Introduction.

Globidium gilruthi, first observed by Moussu and Marotel (1902) and mistaken by them for a developmental stage of *Eimeria faurei*, was rediscovered by Gilruth, in 1910, as a common parasite of sheep in Australia. Later in the same year it was studied by Chatton, who found that it was present in almost all sheep and goats slaughtered at the Paris abattoirs. Chatton gave a detailed description of the morphology of the cysts as they may commonly be found in the walls of the abomasum ; that is, in the late stages of nuclear proliferation and spore development. He further created a new genus *Gastrocystis* using this parasite, to which he gave the specific name *gilruthi*, as the type.

In 1920 Nöller collected together data relating to this and other similar parasites which had been described from various hosts and given a number of generic titles, and included them all in the genus *Globidium*, the type species of which, *Globidium leuckarti* Flesch 1883, occurs in the intestinal epithelium of the horse. In 1925 the present writer published a series of observations on *G. gilruthi*, dealing with the incidence of the parasite in Britain, and confirming Chatton's description of the morphology, but, being unfortunately unaware of Nöller's publication, used Chatton's generic title, *Gastrocystis*. Although at this time many heavily infected

portions of tissue were sectioned and examined in the hope of finding earlier stages of development than those already described by Chatton, only these advanced phases of development were discovered.

The object of the present paper is to give some description of the young stages of *G. gilruthi* and to compare the development of this species with a parasite previously described from the intestinal epithelium of Bennett's Wallaby and believed to be *Globidium mucosa* (Blanchard, 1885), Nöller, 1920. The material was obtained from a young goat slaughtered at the Institute's Field Station, the abomasum of which was seen to be very heavily infected with advanced stages of *G. gilruthi*.

Fig. 1.—Epithelial cell of abomasum of goat containing uninucleate stage of *G. gilruthi*. (× 2,000.)

Portions of this tissue which were sectioned and examined proved also to contain a number of earlier stages than have previously been described. Giemsa stain was used in the preparation of all the material described below.

The Uninucleate Stage.

The earliest stage found, consists of a small, faintly staining cytoplasmic mass, spherical or somewhat irregular in outline, containing a single large, and frequently irregularly shaped nucleus. The cytoplasmic body measures from 6μ to 8μ in diameter, and the necleus, which stains very intensely, has an average diameter of 4·25μ. These parasites are found embedded in the cytoplasm of epithelial cells, which, except for a slight displacement of the nucleus, which is in some cases slightly indented on the side nearest the parasite, appears to be perfectly normal, both in size and staining reactions. These earliest stages are invariably found to occur in epithelial cells which are directly in

contact with the submucosa. The surrounding tissue gives no indication of a host reaction.

The Young Plasmodium.

No intermediate stages between the small uninucleate form described above and a larger form consisting of a 12-nucleate plasmodium were discovered. This plasmodium, which is irregularly rectangular in outline, is sharply defined from the surrounding cytoplasm of the host cell by a fine limiting membrane. The nuclei which are irregularly

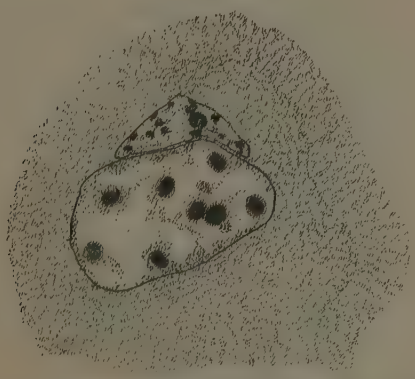

Fig. 2.—Section of 12-nucleate plasmodium of *G. gilruthi* embedded in cytoplasm of enlarged epithelial cell. The cytoplasm of the host cell shows two zones, the outer of which is in process of differentiation into the fringe. (× 2,000.)

scattered throughout the cytoplasm, are ovoid and very deeply staining. They vary slightly in size from 1·25μ to 1·5μ in length by 0·7μ to 1·25μ in breadth. The cytoplasm is granular and rather deeply staining, forming an investing envelope around each of the nuclei and extending outwards from these in the form of an irregular network, to the limiting membrane. The parasite at this stage measures 16μ in length by 9·5μ in width. The host cell, in whose cytoplasm it is embedded, is roughly spherical in shape, and of an average diameter of 28μ.

This enlargement in the size of the parasitised cell is accompanied by an interesting structural change. The nucleus is displaced by the parasite, to which it is closely applied, and distorted into a somewhat crescentic form as seen in section. The cytoplasm is divided into two distinct zones. The inner portion retains the usual finely granular structure and faint blue staining reaction of the cytoplasm of the normal epithelial cell, but this is surrounded by a peripheral layer of 2μ to 4μ in thickness which appears to be in process of transformation into the fringe-like margin characteristic of the older stages of the cyst. This zone can be best described as being radially vacuolated; that is, elongated, very narrow vacuoles extend through it from the external limit of the inner zone towards the outer margin, giving the cytoplasm the appearance of being incompletely divided into radial strands. Further, the straining reaction of this zone differs from that of the inner zone. In the proximal region it shows a blue colouration which is, however, much fainter than that of the main cytoplasmic mass, and this shades gradually to a faint but distinct pink at the outer margin. As has been previously noted the fringe-like outer layer of the wall of the mature cyst shows a marked affinity for eosin dyes. Thus the gradual change in the staining reaction of the developing fringe, and its origin by the reorganisation of a definitely marked-off zone of cytoplasm, and not, as was suggested in a previous publication by the outgrowth of rhizopodal processes, is of considerable interest.

Further Development of the Plasmodium.

From this stage the plasmodium increases in size and tends to become spherical. The increase in size is accompanied by a rapid proliferation of nuclei which become progressively smaller and are scattered irregularly throughout the cytoplasm. Many of them tend to collect around the inner margin of the limiting membrane, which becomes thicker and more distinct.

Meanwhile the hypertrophy of the host cell continues, and seems, for a time, to outstrip the growth of the parasite. The fringe completes its development and attains a depth of 15μ to 20μ in regions where no pressure is exerted upon it by the surrounding tissue. The remainder of the cytoplasm becomes vacuolated, the vacuoles gradually increasing in size as growth continues. The nucleus increases rapidly and becomes more and more flattened by the growth of the parasite, while one or

more large, deeply staining bodies appear within it. The nature of these has not been ascertained; they may represent aggregations of chromatin or, on the other hand, may be enlarged nucleoli and consist of plastin.

With the continuation of growth, the plasmodium begins to increase more rapidly in size than the containing host cell, and gradually encroaches on the cytoplasm of the latter, which ultimately becomes reduced to

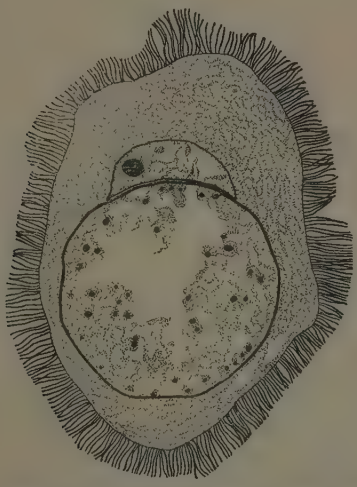

Fig. 3.—Half-grown plasmodium containing many small nuclei and incompletely filling the host cell. Host cell shows distortion of nucleus, vacuolation of cytoplasm and fully developed fringe. (× 500.)

a thin layer forming the inner wall of the cyst. The nuclei of the plasmodium become extremely numerous and very minute, and lie evenly distributed throughout the granular cytoplasm.

In the surrounding tissue changes also take place which become more marked as development proceeds. While the actual parasite is still only about 20μ in diameter, the host cell measuring from 30μ to 70μ in diameter, an infiltration of leucocytes is seen which gradually becomes more and more marked and is often accompanied by some œdema and petechial hæmorrhages.

After the enlargement of the plasmodium has resulted in the complete filling of the host cell, and nuclear proliferation has reached an advanced stage, a series of changes begins to occur preparatory to spore formation. These changes, together with the development and morphology of the spores, have already been described both by the present writer and by Chatton, and are therefore not dealt with here.

The Position of the Cyst within the Mucosa.

During the examination of the material from which the above observations were made, it was noticed that the different stages of development of the cysts consistently occupy different positions in the mucosa. Thus, as has already been noted, the earliest stages are present only in the deepest layer of the epithelium, directly in contact with the submucosa. Half-grown plasmodial stages extend outwards from the submucosa, from which they are separated by a tightly packed mass of leucocytes, and occupy from half to two-thirds of the depth of the mucous layer. In the later stages the cysts are found to be progressively more superficial in position, and, when fully mature, are separated from the external surface of the mucosa by a single layer of cells only, and from the submucosa by the greatly enlarged leucocytic mass, and also, in some cases, by a certain amount of normal epithelial tissue. The final dehiscence of the cyst, by the rupture of the covering layer of cells and the portion of the cyst wall thus exposed, allows the spores to be liberated into the lumen of the abomasum.

Although the mode of infection remains unknown, these changes in the position of the parasite during growth appear to be of some significance, and the origin of the cysts in the deepest layer of the mucosa suggests that the life-cycle in the vertebrate host is not wholly confined to the abomasum.

Comparison of *G. gilruthi* with *G. mucosa*.

In the uninucleate stage *G. gilruthi* exactly resembled *G. mucosa* in the structure of the actual parasite, but, owing to the larger dimensions of the cell attacked, less distortion was caused by the former than by the latter species.

No uninucleate stage of *G. gilruthi* measuring more than 7μ in diameter was observed, and there were no intermediate stages between this and the 12-nucleate plasmodium described above in the material examined.

The initial changes in the host cell caused by this species cannot, therefore, be compared with those caused by *G. mucosa*. In the binucleate stage of the latter, however, portions of the fringe were fully developed and the body of the parasite was larger than that of the 12-nucleate plasmodium of *G. gilruthi*, the host cell of which was merely beginning to show indications of the development of the fringe. Throughout the later stages of development the fringe of *G. mucosa* remained scanty and fragmentary, and assumed rather the aspect of a ragged border than a well developed fringe-like outgrowth.

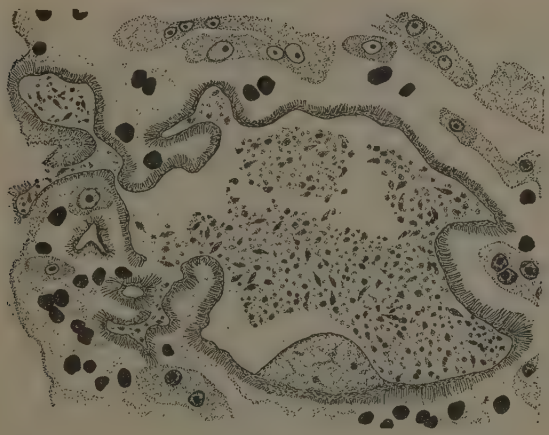

Fig. 4.—Mature cyst in process of dehiscence showing escape of spores into the lumen of the abomasum accompanied by crumpling of the cell wall. (× 500.)

During the growth of the plasmodium the two species also differed in actual structure of the parasitic body. Whereas in *G. gilruthi* the nuclei were irregularly scattered throughout a comparatively small cytoplasmic mass, in *G. mucosa* the cytoplasm at an early stage almost filled the host cell, and the nuclei were, until an advanced stage of development, collected together within a small central mass of cytoplasm which was coarser in structure than that composing the remainder of the body.

The early stages of spore formation and mature spores were both lacking from the material of *G. mucosa* available to the writer, but, from the arrangement of the developing spores in an almost mature cyst it seems probable that these arise singly and not in radial groups as in *G. gilruthi*. Finally the size of the cysts of the two species show a great discrepancy, those of *G. gilruthi* measuring up to 0·9 mm. in their longest diameter while the largest found by the writer in the material of *G. mucosa* measured only 0·04 mm. in length.

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A New Species of *Eimeria* parasitic in a Millepede.

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During a series of investigations concerning the gregarine parasites of common British myriapods a considerable number of examples of *Tachypodoiulus niger* obtained from various districts were dissected and examined. With a view to obtaining cysts of any gregarines that might be present in the gut, the millepedes were isolated for several days, and the fæces examined before dissections were carried out. In examining the fæces of one individual which had been obtained from a wood on the premises of the Institute's Field Station, a large number of oöcysts belonging to the genus *Eimeria* were discovered. Subsequent examination of the alimentary canal revealed the presence of numerous oöcysts in the contents of the hind-gut, while none were present in the fore- or mid-gut contents.

In the passed fæces a few oöcysts were present in an undifferentiated state, with the granular cytoplasmic contents completely filling the internal cavity. These were apparently unfertilised as no further development took place throughout a period of eight days during which they were kept under observation. A single oöcyst was found in which the contents were rounded into a spherical central mass, but the remainder contained fully developed sporocysts in the majority of which the sporozoites appeared to have completed their development.

Oöcysts obtained from the gut contents, were, with the exception of a few which were unfertilised, also at an advanced stage of development, showing sporocysts with either partially, or, more seldom, completely differentiated sporozoites. No sporoblastic stages were discovered in any portion of the alimentary canal.

The oöcysts varied from ovoid to somewhat pyriform in shape, the majority being ovoid. In size they varied from 19μ by 12μ to 40μ by 25μ, the average size being 29·6μ by 17·7μ.

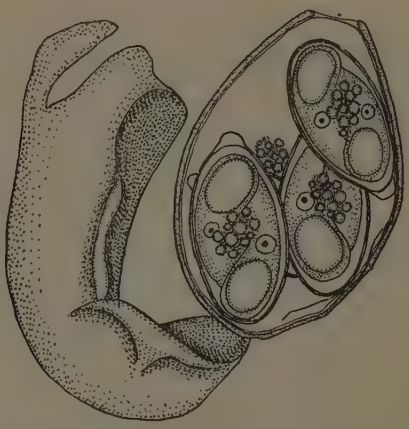

Fig. 1.—Fully developed oöcyst extruded from outer coat, showing slight crumpling of inner wall. (× 2,000.)

The cyst wall was thick and double in structure, the outer, thicker layer being of a faint brownish shade, while the inner layer was clear and colourless. The total thickness of the wall was about 2·25μ of which the outer layer represented 1·5μ and the inner 0·75μ. In the fæces many of the cysts showed the outer layer scaled away in varying degrees, and, where this had occurred, the more delicate inner wall showed a tendency to collapse inwards, giving the cyst a somewhat crumpled appearance. That this was not entirely due to desiccation was shown by the fact that it occurred in moist, newly passed fæces,

as well as in those that were kept artificially moistened, although it was much more marked when the containing fæces were allowed to become dry. The micropyle was distinct and conspicuous, measuring 4μ to 5μ in diameter. In the more pyriform oöcysts it was situated at the narrower pole. An oöcystic residuum, while invariably present, showed great variation in quantity.

The sporocysts were of an elongated lemon shape, with a clear, hyaline rostrum at the narrow end. The wall of the sporocyst was distinctly double contoured. A large sporocystic residuum was constantly present.

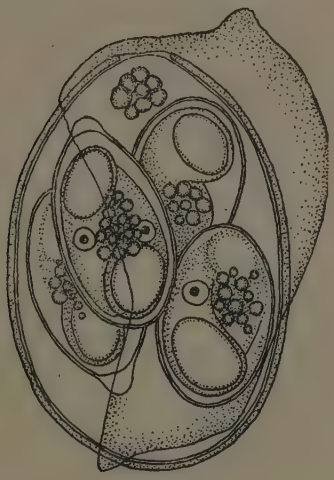

Fig. 2.—Large fully developed oöcyst as found in fæces, still partially enveloped in outer wall. (× 2,000.)

In size the sporocysts varied from 8μ to 16μ in length by 5μ to 9μ in breadth. The sporozoites were of the usual type, and lay curved around the residual body. Each contained a large distinct nucleus with central karyosome.

Two other specimens of *Tachypodoiulus niger* which were also infected with this parasite were subsequently discovered. It is interesting to note that both of these were obtained from the same locality as the first, one from the same wood, the other from a spinney a few hundred

yards distant. Although a considerable number of specimens collected in Kent and in other parts of Hertfordshire were examined, no infection was discovered among them. The parasite appears therefore to be very local in its distribution.

A dissection was made of the alimentary canal of one of the infected specimens and this was embedded and sectioned throughout in the hope that the remainder of the life-cycle might be discovered. It was found, however, on examining this material, that, although oöcysts were numerous in the contents of the hind-gut, no developmental stages were present in the cells of the wall. This seems to indicate either that the infection is of a very transitory nature and dies out after one or more generations of oöcysts have been produced, or that the seat of infection is in some organ other than the epithelium of the main portions of the alimentary canal. That it is a true parasite of the millepede host, and not merely an accidentally ingested organism, is concluded from the fact that, although a great number of other myriapods including *Glomeris* sp., *Geophilus* sp., *Polydesmus* sp. and *Lithobius* sp. were examined which had been obtained from the same small pile of decaying wood chips as two of the three infected *Tachypodoiulus*, none of these were found to harbour any oöcysts.

This parasite is believed to be hitherto undescribed and to constitute the first record of a species of the genus *Eimeria* occurring in a millepede host. The name *Eimeria ekdysios* n. sp. is therefore suggested for it, with the following diagnostic characters based on the morphology of the oöcysts.

Eimeria ekdysios n. sp. Oöcysts ovoid or pyriform measuring 19μ to 40μ in length by 12μ to 25μ in breadth, fully developed when passed in the fæces, with a double wall, the outer layer of which is brownish in colour and peels off after leaving the body of the host. Sporocysts 8μ to 16μ in length, tapering slightly to a hyaline knob at one end.

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On a Nodule-forming Parasite from the Skin of a Newt.

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In examining for helminthic parasites a number of common newts, *Molge palmata* and *M. vulgaris* from the Aberystwyth area, Dr. E. A. Lewis discovered one individual (*M. palmata*) the skin of which was affected with whitish nodules. These nodules varied in size up to 2 mm. in diameter and were irregularly distributed over the body, occurring singly or in small groups on the back and sides, at the bases of the limbs, and on the tail. On separating the skin from the underlying tissues it was found that the nodules were formed by whitish jelly-like masses embedded in the subepidermal tissues. They were surrounded by a fine membrane in which no nuclei could be observed.

When removed from the tissues of the host and crushed between slide and coverslip, the nodular masses were found to be composed of a jelly-like matrix containing ovoid bodies, also of a clear hyaline consistency but which had the appearance of being composed of a firmer substance than the surrounding matrix. Each of these ovoid bodies contained one or two irregularly shaped, highly granular refractive masses in which no definite structure could be made out, but which apparently represented nuclei. Larger bodies of somewhat angular shape were also present, the contents of which had divided into two, three, or four masses, each with a granular refractive nucleus. In addition to these forms small aggregations of spore-like bodies were

also present. The majority of these consisted of mulberry-like masses of small, rounded bodies, clustered together and surrounded by a fine membrane. The spore-like bodies composing these groups varied both in number and size and each contained a distinct spherical nucleus with central karyosome. In some of the groups, which were apparently at a later stage of development, the investing membrane was lost, the spores were particularly distinct in outline, as though each had secreted an outer covering, and were, in addition, drawn out into a slight point at the free outer margin.

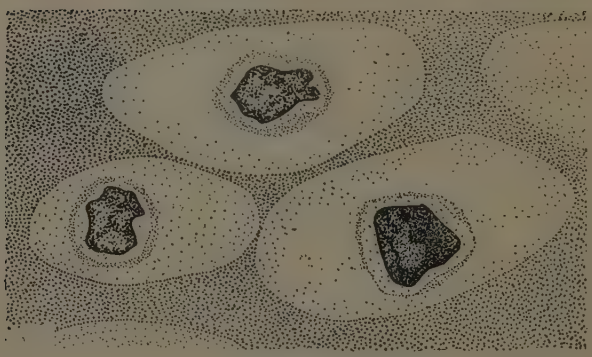

Fig. 1.—Ovoid uninuclear bodies embedded in the matrix. (× 2,000.)

Portions of the body wall containing the nodules were sectioned, and the distribution and development of the forms described above were thus observed. The centre of the nodular mass consisted of a faintly staining, homogeneous substance, within which the ovoid uninuclear and binuclear forms were embedded. Surrounding these was a

zone containing segmented stages only. Here no matrix was visible, and the cells were closely packed together causing mutual distortion into angular quadrilateral forms. The outermost layer contained scattered groups of spores at various stages of development.

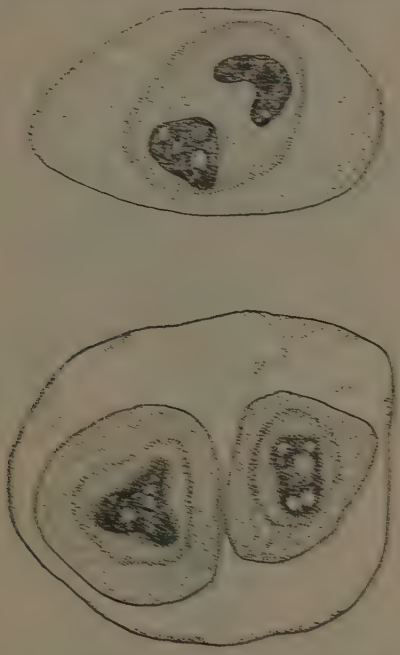

Fig. 2.—Early stage of division. Nuclear division has not yet been followed by the division of the granular cytoplasm. (× 2,000.)

Fig. 3.—Parent cell containing two daughter cells. (× 2,000.)

The centrally placed ovoid bodies varied in size from 17μ by 10μ to 30μ by 15μ. They consisted of very faintly staining, almost homogeneous cytoplasm containing an irregularly shaped, deeply staining nuclear mass. Around the nucleus and separated from it by a space of about 2μ, was a ring of slightly granular cytoplasm from 1μ to 2μ in thickness.

A fairly complete series of developmental stages between this uninuclear form and the spore masses was discovered. The life-cycle up to spore development appears to be as follows.

The granular cytoplasmic ring around the nuclear mass becomes both larger and more distinct as the uninuclear form increases in size.

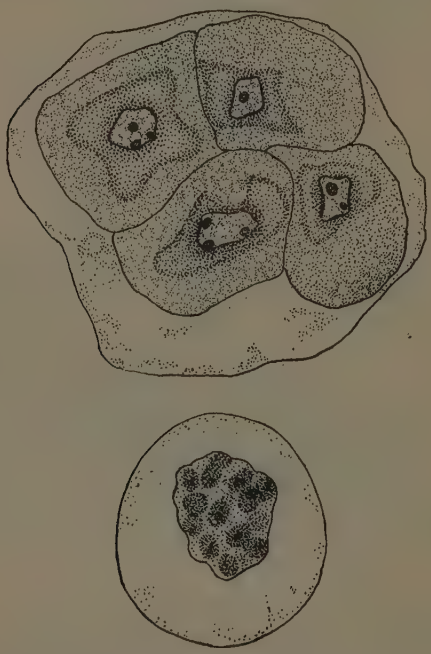

Fig. 4.—Parent cell containing four daughter cells in one plane. (× 2,000.)
Fig. 5.—Early stage of spore formation. (× 2,000.)

Nuclear division takes place, the daughter nuclei separating within the cytoplasmic ring, which also divides. Two separate bodies are thus formed within each ovoid parent cell, and these become sharply defined from the surrounding homogeneous cytoplasm, the outer layer of each forming a fine membrane which stains readily. A further differentiation

then takes place within the cytoplasm of the daughter forms, a new granular zone arising around the nucleus and again becoming more distinct as development proceeds. A second nuclear division, accompanied by a division of the cytoplasm, then takes place, resulting in four daughter cells within the parent cell. Frequently the second divisions are not completed simultaneously, as, in many cases, three bodies, one large and two smaller, were found within the parent cell. The four daughter cells thus formed are usually arranged in a tetrad formation, and only in exceptional cases lie in a single plane. Each of them consists of a granular cytoplasmic mass enclosed within a deeply staining membrane. The nucleus, eccentrically placed, can now be seen to possess a definite nuclear membrane. Surrounding the nucleus, a highly granular ring of cytoplasm still persists.

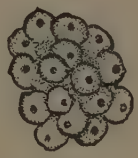

Fig. 6.—Small group of large spores. (× 2,000.)
Fig. 7.—Small group of small spores. (× 2,000.)
Fig. 8.—Fully developed spores showing cuticle. (× 2,000.)

Spore formation now takes place. Early stages were not seen, but only the nucleus and the granular ring of cytoplasm appear to be involved in this process. The outer zone of cytoplasm of the daughter cell loses it staining reaction and gradually, together with the parent cell, breaks down, leaving a mulberry-like body of spores enclosed within a fine membrane. The groups of spores vary from 12μ to 20μ in diameter. while the spores themselves vary from 1·25μ to 3·5μ in diameter. All the spores present in a single group are of approximately equal size. With the development of the cuticle and the disappearance of the investing membrane the spore masses lie free at the periphery of the nodule.

The nature of this parasite has not been determined. Cellulose tests were carried out both on the sections and on the nodular masses extracted from the tissues, but these gave negative results. It seems, however, probable that the parasite is a fungoid growth and not a protozoan. The infected newt was preserved whole in formalin, and the lack of nuclear structure in the early stages of development may therefore have been due to imperfect fixation. Nothing is known regarding the dispersal of the spores or the mode of transmission.

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1922.—(*a*) A Simple Method of Experimentation for Skin Infection with Hookworm Larvæ.
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(*b*) On the Susceptibility of Clover and some other Legumes to Stem-disease caused by the Eelworm, *Tylenchus dipsaci, syn. devastatrix, Kuhn.*
Journal of Agricultural Science. Vol. XII., p. 20.

(*c*) Observations on the Ensheathed Larvæ of some Parasitic Nematodes
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(*d*) The Eelworm in Paper-hanger's Paste: *Anguillula rediviva* (Linnæus, 1767.)
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1923.—(*a*) Experiments on the Feeding of Embryonated Eggs of *Ascaris megalocephala* to Domesticated Animals.
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(*b*) Quiescence and Reviviscence in Nematodes, with special reference to *Tylenchus tritici* and *Tylenchus dipsaci.*
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